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Toxicity of soil fumigants in relation to seed dormancy

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TOXICITY OF SOIL FUMIGANTS IN RELATION TO SEED DORMANCY

by

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INTRODUCTION

Traditionally, weed control practices have emphasized the control of weeds after germination and emergence of seedlings. Destruction of weed seed in the soil has received little attention except in seedbed fumigation for high value crops. Fumigation as a method of weed control has provided information, on an empirical basis, for the appropriate rates of application of various fumigants needed to control weeds on particular soils and under specific conditions. Furthermore, these techniques have emphasized the control of non-dormant, germinable weed seed with little consideration for long term weed control by the elimination of both non-dormant and dormant weed seed.

Seed dormancy is a major adaptation of annual weeds which permits them to survive and flourish in spite of many agronomic practices designed to favor the establishment of the crop. Thus, propagules of weed species may remain dormant or in the non-growing state for extended periods. During this time, varying percentages of seed may encounter that combination of environmental conditions which results in the termination of dormancy. An understanding of the specific conditions under which dormant seed may remain viable, of the narrow range of environmental conditions under which they lose dormancy, and of their susceptibility to soil fumigants under these conditions, is basic to the development of weed control practices designed to eliminate weed seed in the soil.

The present investigation was directed to an evaluation of the toxicity of selected soil fumigants to dormant and non-dormant seed of selected annual weed species. A second aspect of the study included a

preliminary attempt to characterize weed seed viability and dormancy under field conditions. The several fumigants used were chosen for their suitability of handling and on the basis of proven effectiveness in seedbed fumigation. Abutilon theophrasti Medic., Polygonum pensylvanicum L., Setaria lutescens (Weigel) F. T. Hubb., and Setaria faberi Herrm. were used because they met the major requirements for suitable experimental material for the study. Adequate supplies of both dormant and non-dormant seed were available, methods were known for breaking dormancy, distinct types of seed dormancy are represented, and the species are serious weed pests of the region.

LITERATURE REVIEW

Soil fumigation for the control of soil pests has been known for about 100 years. However, it is only within the last two decades that extensive research has been conducted into properties of soil fumigants as they pertain to basic toxicity, mode of action, and function in soil. Historically soil fumigants were used to control pathogenic microflora associated with soil. In these studies it was noted often that weed populations were reduced following fumigation. This led to further studies into the relationship of the physiological and environmental condition of the seed and fumigant toxicity. Toxicity may depend on the life stage of the pest being treated as well as any biological variation existing within a group of organisms. Basic toxicity of a fumigant in relation to its rate of application greatly influences pest control under normal conditions. Obviously, many factors modify the results obtained with soil fumigation; an appreciation of these factors is essential for effective control programs.

Observed Toxicity under Field Conditions

Toxicity of soil fumigants under field conditions depends upon many factors related to the soil and fumigant used. Studies on physical and chemical properties of the movement through, or reaction of, fumigants with soils or soil constituents has revealed the complex interrelationship between soil and fumigant. With optimum or ideal conditions complete soil fumigation results from radial diffusion of fumigant from injection points through a transition stage to a reasonably uniform distribtion of

the fumigant in the soil. Hemwall (18) stated that optimum fumigant properties are (1) low chemical reactivity with soil components, (2) low diffusibility, (3) high water solubility, (4) low vapor pressure and (5) low absorbability by soil components. Optimum soil properties included (1) low organic matter content, (2) low adsorptive capacity for fumigants, (3) low continuous air space, (4) ideal soil temperature depends on fumigant, but in general the higher the temperature the more effective the fumigant, (5) soil should be well worked for best results, and (6) some soil moisture is desirable, but less than field capacity. At low levels of moisture the fumigant is lost too quickly while at field capacity there is not enough soil pore space for free movement of the fumigant.

Early workers were not so much interested in these properties as such as they were in the end result of soil fumigation. Prominent among the early fumigants was carbon bisulfide and according to Young (58) no weed control resulted with applications of 1000-3000 lb/acre. Crowther and Richardson (9) studying the effects of decomposition of calcium cyanamide in the soil and its effects on germination, found that toxicity to germinating seed was caused by cyanamide. Toxicity was reduced rapidly as the time interval between applying the calcium cyanamide and sowing seed increased and was proportional roughly to the amount of cyanamide present during a relatively short time interval after sowing. Combinations of cyanamide and sodium azide, allyl alcohol and ethylene dibromide were shown to give good weed control by Clayton et al. (6). He also found that calcium nitrate and potassium nitrate were effective weedicides, when applied at rates of 1 lb/yard^2.

Chloropicrin was the first true soil fumigant investigated extensively. In an extensive study of chloropicrin as a soil fumigant, Stark (43) found that the smaller the size of soil particle the greater the amount of chloropicrin adsorbed; degree of aggregation of soil particles had little or no effect on amount or rate of adsorption of chloropicrin. Increasing the temperature resulted in a linear decrease in the amount of chloropicrin adsorbed and the presence of moisture in the soil decreased the amount adsorbed. High organic matter content also decreased adsorption by soil, while pH did not affect adsorption. Godfrey (16) obtained 100 percent control of nut grass (Cyperus rotundus) with chloropicrin treatments of 560 lb/acre with plots covered with paper. Treatments of 400 lb/acre with cover or no cover gave only about 50 percent control. Young (58) reported Johnson grass (Sorghum halepense) and crab grass (Digitaria sanguinalis) usually were controlled at rates of 250-450 lb/acre when plots were covered with glue-coated paper for 3 days following fumigation. However, weed seeds were controlled poorly where the soil was dry at time of treatment.

In 1948 Newhall and Lear (34) reported the effectiveness of methyl bromide in control of weeds in greenhouse flats. Sandy loam, 1 foot thick, was treated with 60 ml in each hole with holes 12 inches on center, 5 inches deep and covered for 48 hours. Treated flats had a third as many weed seedlings and these were all clovers, whereas untreated flats contained purslane and many grasses. Freeman (14) demonstrated that for best results with methyl bromide soil temperature should be above 70°F, however, between 60-70°F good weed control was obtained while very poor control occurred between 40-50°F and no control was observed below 40°F.

Under favorable temperature, plots covered for only 6 hours did not result in control of weeds, whereas 18 hours of covering gave good results. He also showed that good soil tilth and soil moisture between 11-24 percent were most important for effective fumigation. Even under optimum conditions, seed of annual morning glory, white clover, garden mallow and velvetleaf were not killed. Dieter and Coulter (12) used asphalt-laminated paper to cover plots after treatment and also found very poor weed control at soil temperatures below 50°F and that best control was obtained when the soil was moist for several days prior to treatment. Wetting the soil just prior to treatment gave better weed control than dry soil.

Adamson (1) reported 100 percent control on plots infested with field bindweed when fumigated with 1 or 4 lb/ft^2 of methyl bromide. Rates of .5 and .25 lb/100 ft^2 resulted in only 30 and 20 percent control of weed seedlings, respectively. Treatment time was 24 hours with soil temperature from 60-70°F and the soil below field capacity. Hill, Klingman and Woltz (19) reported that fall or spring application of methyl bromide at a rate of 1 lb/100 ft^2 and covered for 48 hours controlled seeds of 16 species mixed into the top 2 inches of soil with the exception that legumes were not controlled in fall treatment because of dry soil. Under the same conditions allyl alcohol did not control seed of Jerusalem oak either in fall or spring application but did control the legumes.

DeFrance, Bell and Odland (11) applied allyl alcohol at rates of 2, 4, or 6 lb/1000 ft^2 and obtained weed control after 2 weeks at all rates and over 90 percent control at 8 weeks. All rates were toxic to radish

seed planted at weekly intervals up to 6 weeks after fumigation and none were toxic after 6 weeks. All rates were toxic to colonial bent seed for 2 weeks but none after this period; toxicity to rye grass increased as rate of application increased.

Skoog (42) found that the number of legume weeds were not reduced by fumigation with Vapam, allyl alcohol, or methyl bromide. White clover was the principal legume present. Methyl bromide gave the most consistent control of weeds, whereas allyl alcohol and Vapam gave inconsistent results. Lloyd (31) has shown Vapam to be phytotoxic at concentrations of one part per million in the soil atmosphere. Hunnam and Waddington (20) demonstrated that .05 parts per million in the air about tomato plants for 2 days caused serious stunting and malformations. Vorlex (methyl isothiocyanate and chlorinated C_3 hydrocarbons) at rates of 58 gal/acre resulted in good control of Panicum texanun and Digitaria sanguinalis according to Young (57).

Toxicity Observed in Laboratory Experiments

Experiments conducted in the laboratory enable the investigator to obtain more precise information on conditions affecting toxicity and to gain a better understanding of the basic toxicity of a fumigant. Jacks (21) mixed seed and soil together in containers to which was added either a fumigant or steam; the containers were than sealed. Seed were removed 48 hours later and germination determined. His results showed that steam destroyed seed more effectively than any of the fumigants. Clover and grasses proved highly resistant to fumigation and while chloropicrin and dichloropropene-dichloropropane mixture were most effective, they did not

completely inhibit germination of seeds.

Youngson, Baker and Goring (59) mixed oats in the soil before fumigation and found that toxicity of chloropicrin and methyl bromide in sealed containers increased with increased temperature, moisture, and exposure period and decreased with increased organic matter content of soil. They also showed that gravity had no effect on diffusion; the fumigant presumably moved by random molecular diffusion rather than mass flow of vapor. With increased rate of application from 25-800 lb/acre, depth of kill of oat seed increased from 6 to 21 inches with chloropicrin and rates of methyl bromide from 100-800 lb/acre killed seeds at depths of 9 to 27 inches.

Pieczarka and Warren (36,37,38) placed dormant imbibed tomato seed at various depths in soil cubes and determined the toxicities of several fumigants. In these experiments dormant imbibed seed were germinable seed that were allowed to imbibe water but held at such a low temperature as to arrest germination. Vapam at rates of 350 lb/acre in sand had almost complete area of 90 percent kill as did 700 lb/acre in muck. Maximum diffusion of Vapam in muck required 120 hours and in sand only 48-72 hours. Allyl alcohol and 1-3 dichloropropene did not give as much area with 90 percent kill as did Vapam. When dormant imbibed seeds were exposed to various concentrations of fumigant and for different time periods it was shown that as time exposure increased kill of seeds increased and this generally held for concentrations also. Allyl alcohol was the most toxic with .35 mg/l and 1 hour exposure resulting in 100 percent kill of pigweed and a 4 hour exposure at this concentration resulted in 100 percent kill of tomato seeds. Vapam showed 100 percent

kill at concentrations of 88-220 mg/l with 8 to 10 hours exposure time.

Dry seed, with moisture content below ten percent, treated with methyl bromide at rate of 4 lb/1000 ft^3, at 70°F for 12 hours showed very little effect of treatment as determined by germination according to Lindgren, Vincent and Krohne (30). Alfalfa, sunflower, milo, and sudan showed no effect of methyl bromide when moisture content was below eight percent, however, milo, sudan, and sunflower showed great reduction in germination when moisture content was above 12 percent. Strong and Lindgren (44,45,46,47,48) in a series of papers on the effects of methyl bromide and hydrocyanic acid on seeds found that the moisture content critical for maximum toxicity varied with the species involved. Wheat had a critical moisture percentage between 10 and 12 percent, corn was 12 percent, oats approximately 12 percent, rice 10 percent, and barley showed no increase in kill as moisture content increased over range of 8-14 percent. All seeds showed increased kill with increase of temperature over range of 50-90°F and increased exposure time. Methyl bromide with two hour exposure at 5 lb/1000 ft^3 and 70°F resulted in very little kill while 8 hour exposure gave some kill and 24 hours gave almost complete kill to all seeds when moisture content was above the critical level. In experiments using two fumigation treatments the second treatment increased percent kill only in instances where the first treatment was effective.

Results reported by Cobb (7) showed that germination of lettuce seed treated with methyl bromide at a rate of 250 lb/1000 ft^3 was reduced only two to four percent when at 4.3 percent moisture, but 100 percent when at eight percent moisture. Sorghum treated at the same rate had

25 percent reduction at 6.6 percent moisture and 100 percent kill when at 9.6 percent moisture.

Gammon (15) demonstrated that very little damage occurred to dry vegetable seed fumigated with methyl bromide at rates of 1 or 2 lb/1000 ft^3 for 24 or 35 hours. However, seed held at 100 percent relative humidity for 1 week and where moisture percentage reached 11.9 percent a 50 percent reduction in germination occurred. Fumigation of crop seeds with chloropicrin at a rate of 3 cc/14 liter for 24 hours showed some seed responded to relative humidity differences during treatment and others did not. With seed that did respond to relative humidity, moisture content of the seed was important only when treated at low relative humidities and not when treated at high relative humidities. Relative humidity at time of fumigation was found to be more important than relative humidity 24 hours prior to fumigation. Somewhat different results were obtained by Bruch and Koesterer (3) who treated dried spores of Bacillus subtilis with 1250 mg/l propylene oxide at 99°F and 80 percent relative humidity and found time for 90 percent kill to be 1 hour whereas at 25 percent relative humidity the time was only 40 minutes.

Results of seed fumigation studies demonstrate that the margin of tolerance is dependent upon the complex interactions of several variable factors including, (1) fumigant dosage applied, (2) moisture content of seed, (3) exposure times, (4) kind of seed, (5) period and conditions of storage after fumigation, (6) temperature during fumigation, and (7) condition and age of seed. These interactions do not yield the same result with all fumigants and the complexity varies with mode of action of a particular fumigant. Little is known about mode of action of

fumigants and data in the literature are often conflicting.

Most extensive work on this problem has been done with methyl bromide. Lubatti and Harrison (32) studied the sorption of methyl bromide by wheat and found that sorption increased as moisture content increased; increase in temperature from 55° to 82°F resulted in 1.5X more sorption; and sorption of fumigant and wheat did not reach equilibrium for many days, long after normal treatment periods. Methyl bromide sorption was slow, approximately 3-4 mg/100 seed after 24 hours and 75 percent of that sorbed was released in 2 hours of aeration. In onion seeds Lubatti and Smith (33) showed that the rate of sorption appeared to be governed by diffusion into the seed and greater sorption occurred if seed were exposed to a constant concentration of fumigant. They suggested that the reason for greater sorption at increased moisture content was greater permeability for fumigant entry, more free water available for solubility of fumigant and greater chance for chemical reaction. Possible chemical reaction could occur with disulphide bonds or products of hydrolysis of glycosides.

Lewis (29) demonstrated that methyl bromide may react with sulfhydryl groups and enzymes known to depend upon sulfhydral groups for activity were irreversibly inhibited when exposed to methyl bromide. Most recent evidence by Winteringham, Harrison and Bridges (54) and Bridges (2) showed that methyl bromide sorbed by wheat under conditions of fumigation underwent chemical decomposition with formation of inorganic bromide and a series of methylated derivatives. The protein fraction of wheat accounted for 80 percent of the methylation and the methylation reaction

was highly specific, being N-methylation of the imidazole ring of histidine residues. This methylation of histidine produced the toxicity. Cobb (8) lent support to this hypothesis by showing that the amount of bromine present in the seed after fumigation had no bearing on germination. Viable embryos one year after fumigation had 263 parts per million bromine, whereas before fumigation only traces were present.

Mechanism of Vapam toxicity is not well understood. The toxic substance appears to be methyl isothiocyanate, a decomposition product of N-methyl dithiocarbamate, but N-methyl dithiocarbamate has been shown to be toxic under certain conditions. The mode of action of methyl isothiocyanate or N-methyl dithiocarbamate is not known. Respiration, permeability and survival studies on Rhizoctonia solani have placed the fumigant in a group of fungicides with non-specific toxic effects. Wedding and Kendrick (52) suggested the mode of action is a reaction with some constituent of the cell membrane that contains sulfhydral groups in such a way as to produce a physical change in membrane structure and alter or destroy its effectiveness as a barrier to either loss of essential cell constituents or entry of the toxicant into the cell.

Legator and Racusen (28) determined that the toxicity of allyl alcohol was due to an in vivo conversion of allyl alcohol to acrolein. Allyl alcohol is an excellent substrate for alcohol dehydrogenase and acrolein, the product of allyl alcohol oxidation, is a potent sulfhydral reagent inhibiting any enzyme dependent on sulfhydral groups for activity.

Seed Dormancy and Germination

The use of soil fumigation as an effective weed control practice requires a good understanding of the dormancy patterns of the major weed species under field conditions.

Seed dormancy is a major adaptation of annual weeds which permits them to survive and flourish in spite of the many agronomic practices designed to favor the establishment of the crop. In temperate zones it is a survival mechanism which prevents the fall germination of newly matured seed of species which are not winter hardy. Dormancy may be attributed to a number of biochemical, physiological and physical factors. Only dormancy patterns relating directly to weed species used in this study will be reviewed.

Dormancy with seed of Abutilon theophrasti was studied by LaCroix and Staniforth (27) who showed that dormancy was maintained primarily by impermeability of the seed coat to water and secondly by an inhibitory system which may further prevent germination after water entry. Termination of dormancy was attributed to a complex series of physical and chemical changes which resulted ultimately in germination. Seed of Abutilon theophrasti develop a thick-walled palisade layer which completely encloses the seed except for a slit-shaped opening in the chalazal region. The opening is covered by a cap of funicular tissue which closes over the chalazal opening when the seed is in a moist environment. This opening was found to be activated by changes in moisture conditions external to the seed, however, tests showed that little or no moisture entered through the chalazal slit. Importance of the opening maybe in loss of moisture during seed maturation and drying, resulting in seed

coat rupture which terminates dormancy.

Nieto (35) found at least two mechanisms operative in the maintance of seed dormancy in Setaria lutescens. One was conditioned by the caryopsis, the other by the lemma and palea. Caryopsis dormancy was terminated readily by the combination of low temperature and high moisture but seed after-ripened in this manner did not germinate appreciably until lemma and palea were removed. Dormancy imposed by the lemma and palea appeared to result from a combination of effects, including impermeability to water, possible mechanical construction of the caryopsis, and possibly inhibitors in the hull. Under natural conditions in the field, a majority of Setaria lutescens seed were ready to germinate in the spring following the year of maturation.

Evidence for the sequence of events involved in the termination of seed dormancy in Polygonum pensylvanicum is incomplete. Woodcock (56) in 1914 gave an excellent account of the morphology and anatomy of certain Polygonaceae seed and suggested that in Polygonum articulatum and Polygonum scandens the aleurone layer secretes an enzyme which converts the insoluble starch of the endosperm into a form available for the germinating embryo. Ransom (39) studied after-ripening requirements for seed of several species of Polygonaceae. He found that seed of Polygonum pensylvanicum treated for 5 months at 43°-48°F under saturated conditions germinated 84 percent, whereas in dry storage no after-ripening occurred. Justice (23) in a thorough study of dormancy with seed of Polygonum showed that Polygonum pensylvanicum seed after-ripened in water at 36°-39°F for periods of 3 to 36 months reached maximum germination at 10 months and remained at this high level until termination of

the experiment. When the temperature was 48°-52°F the after-ripening was not as complete and when temperature was alternated weekly between 48°-52°F and 64°-70°F essentially no after-ripening occurred. After-ripening was more rapid with seed chilled in water rather than placed between layers of moist cotton, but the degree of after-ripening was the same. In experiments with the pericarp removed the length of the after-ripening period was reduced from 2 to 8 weeks depending upon species. Justice also found that naked seed absorbed water at a greater rate than intact seed the first few days, but after 13 days moisture percentages were equal. Seed of most species showed a decrease in the time necessary for after-ripening with increased age up to 18 months and seed older than 28 months gave very low percentage of germination when after-ripened. Effect of overwintering of seed buried 10 cm and 1 cm showed that seed buried at 1 cm germinated better than did those at 10 cm. These differences varied between 15 percent for seed of Polygonum virginianum and 80 percent for seeds of Polygonum pensylvanicum. Studies of embryo dormancy revealed patterns of after-ripening both similar and dissimilar to intact seed. Studies by LaCroix (26) also revealed this complex system governing germination in Polygonum pensylvanicum. Embryo dormancy and germination inhibitors appeared to be involved in an intricate pattern of interactions which may result in varying degrees of embryo dormancy in the presence or absence of overall seed germinability.

Seed germinability in the field the spring following maturation was reported to be very low by Witts (55). Polygonum persicaria, Polygonum aviculare and Polygonum convolvulus had 4, 4, and 3 percent germination, respectively, over the entire growing season of 1957. The same seed lots

in the greenhouse germinated 21, 11, and 14 percent, respectively. Simmonds (41) in a study of Polygonum persicaria found evidence that seed in soil may remain viable for 20-30 years. Also, seed that do germinate in the spring are conditioned by low soil temperature and saturation. Gas exchange may be limiting for after-ripening in case of deeply buried seed or it may be that the generally higher temperature with increasing depth in soil in winter is partly responsible.

Duvel's (13) experiment on the vitality of buried seed supports the suggestion that deeper burial enhances longevity of seed. Duvel stated that seed are better preserved the deeper they were buried. Temperatures were lower, less alternation in temperature occurred, there was a more uniform moisture percentage, and less oxygen present were all favorable storage conditions. Duvel's experiment was started in 1902 and at that time none of the Polygonum pensylvanicum germinated. A progress report by Goss (17) in 1921 and a final report by Toole (49) in 1946 showed that Polygonum pensylvanicum germinated four percent in 1918 and in 1932, two, one, and eight percent germinated at the 8, 22, and 42 inch depths, respectively, and that no germination occurred after 1940. Seed of Abutilon theophrasti did not germinate until 1908 and maintained 70 percent germination for about 26 years. In 1941 Abutilon theophrasti seed impermeable to water had viability of 38 and 42 percent at the 22 and 42 inch depths. Chepil (4) in his studies of the longevity, periodicity of germination, and vitality of seeds in soil demonstrated that all seed of Setaria viridis in loam and sandy loam soils that would germinate did so the spring following maturation. In clay soils almost 100 percent of the seed would germinate in the first year following

maturation but one percent or less continued to germinate for 5 years following maturation. Chepil (5) concluded from his studies that for seed possessing a relatively long period of dormancy, the deeper the seed were buried in the soil the lower was the emergence of seedlings and higher the number of viable seed. Seed possessing a relatively short period of dormancy, depth of burial had little effect for if buried too deep to emerge they soon lost viability anyway. Nieto (35) reported dormant Setaria lutescens seed buried at 1, 2, 4, and 6 inch depths in the fall attained maximum germination after 8 weeks. Results also indicated that depth of burial was not critical. Germination tests were run with lemma and palea removed.

In 1904 Waldron (50) reported maximum depth of emergence of green foxtail to be 3 inches and that of wild oats 5 inches. Kirk and Pavlychenko (25) in Canada reported wild oat emergence from a depth of 7 inches. King (24) obtained seedling emergence of Setaria faberi from 9.5 to 12 cm. Dawson and Bruns (10) studied seedling emergence of barnyard grass, green foxtail and yellow foxtail and found in the field that all three species emerged from all depths in the range of 0-5 inches. Emergence was greatest from the 1 1/2 inch depth and fewest seedlings emerged from the 0 and 1/2 inch depths. Greatest emergence in the greenhouse occurred from the 1 inch depth and barnyard grass and green foxtail did not emerge from the 5 inch depth.

MATERIALS AND METHODS

Experiments designed to evaluate the toxicity of soil fumigants to dormant and non-dormant weed seed required after-ripening of weed seed in the field and laboratory techniques of embryo culture, seed germination, and fumigation. Fumigants used were Vapam, methyl bromide, allyl alcohol, and propylene oxide. Although propylene oxide is not usually considered a soil fumigant, it was included because of it widespread use in sterilizing and killing seed in the laboratory. Seed of Abutilon theophrasti, Setaria lutescens, Setaria faberi, and Polygonum pensylvanicum were used in this study. Seed of the several experimental species were harvested from wild populations and stored at approximately 40°F until used. Seed lots were designated by year of harvest. The term seed henceforth will be used in a general sense to indicate plant propagules such as seed, spikelet, and achene.

Germination Tests

Two filter papers were placed in the bottoms of standard petri dishes (9 cm diameter). The paper was moistened with 5 ml distilled water and the seed distributed uniformly on the surface of the paper. Germination tests ran for 10 days at 86°F in the dark. Germination tests for Polygonum pensylvanicum were allowed to run for 26 days, the time required for maximum germination, but in no tests did the germination increase over that obtained in 10 days and they too were subsequently terminated after 10 days. Germination counts were made at 2, 5, and 10 days after placing in the germinators. Germination is defined for the

purpose of this study, as that stage of growth when the radicle and epicotyl were 1 cm or more in length. At this stage of growth it was evident that a seedling would develop. Whenever possible, germinability of Setaria lutescens seed was determined with lemma and palea removed and with seed of Abutilon theophrasti and Polygonum pensylvanicum by removal of seed coat and fruit coat, respectively. Growth of isolated embryos was also used as a measure of the germination potential and viability of seed lots.

Embryo Culture Technique

Seed coats of Abutilon theophrasti and Polygonum pensylvanicum were removed by cutting through the coat around the seed periphery with a razor blade. Lemma and palea of Setaria lutescens were removed using a dissecting needle and razor blade. Prior to embryo excision caryopsis or seed was soaked in water for 2 to 4 hours to facilitate removal of the embryo. Scalpels and forceps were dipped in 50 percent ethyl alcohol and flame sterilized prior to use. Embryo isolations were done under a dissecting microscope at 50X magnification. Isolated embryos were surface sterilized in calicum hypochlorite solution for 1 to 2 minutes and then rinsed in sterile distilled water. All manipulations were carried out in a transfer chamber to minimize contamination. Deionized water, used in preparation of culture media, was obtained by passing distilled water through a Barnstead demineralizer. Reagent grade chemicals were used in preparation of media. Embryos were cultured on a semi-solid culture medium, based on that described by Rappaport (40) with some modifications. Since embryos from non-dormant seed grew normally on this medium no

additional modifications of the media were utilized. The following stock solutions were prepared:

Stock solution A

$Ca(NO_3)\cdot H_2O$	23.6 g
KNO_3	8.5 g
KCl	6.5 g
H_2O to 500 ml	

Stock solution B

$NaH_2PO_4\cdot H_2O$	1.35 g
$MgSO_4$	3.02 g
$MnSO_4\cdot H_2O$	.76 g
H_2O to 250 ml	

Stock solution C

A chelated form of iron was prepared using the method of Jacobson (22). The ethylene diamine tetra-acetic acid was obtained from Eastman Chemical Company. One milliliter of the solution provided five parts per million of iron in one liter of nutrient medium.

Basic medium was prepared according to the following schedule:

1. 20 g sucrose was added to 1 liter of water, and the solution was brought to a boil.
2. With constant stirring, 8 g of agar (Difco Special Noble) was added slowly, and the mixture boiled until the agar was completely in solution.
3. The solution was allowed to cool; then 5 ml of solution A, 2.5 ml of solution B, and 1 ml of solution C were added.

4. The medium was poured into embryo culture flasks and autoclaved for 20 minutes at 15 lb. pressure.

After-ripening in the Field

1963 seed of Setaria lutescens and Polygonum pensylvanicum were buried in Clarion-Webster loam on November 16, 1963. Approximately 500 seed of each species were placed in a 2x2x2 inch wire basket and buried at three depths 1, 3, and 6 inches. Seed were also placed on the soil surface with the wire basket inverted over them. Four baskets, one for each depth, were placed together stepwise in the soil. Four replications with 32 sites, four baskets at each site, were used. Half of these sites were covered with clear plastic in such a manner as to allow air movement but keep precipitation off the seed and the other half were exposed to natural field conditions. At two week intervals beginning December 4, four dry and four wet (natural conditions) sites were excavated and seed tested for germination, viability, susceptibility to fumigation, moisture content, and rate of water uptake.

Fumigation

Fumigation treatments involved placing petri dishes with covers removed in wide-mouth one gallon jars, the fumigant added in the proper amount, and containers placed for the duration of treatment in a controlled temperature chamber. After fumigation the jars were vented and petri dishes removed. The seed were exposed to air for 2 to 4 hours before placing in germinators.

Neoprene gaskets were placed inside the jar covers and two 3/8 inch copper tubes were soldered on the covers so that they extended 1 inch

above and below the cap. Tygon tubing was fitted over the copper tubing on the outside and closed with a crew clamp to obtain a tight seal. A piece of polyethylene with a hole in the middle was placed over the mouth of the jar and the cap screwed tight. This additional gasket helped to obtain a better seal between the cover and the mouth of the container. A piece of cheesecloth was wrapped around one copper tube extending into the container and tied in place. When the material was injected into the jar, it would first be absorbed on the cloth, and then allowed to vaporize inside the jar.

It was feasible to inject Vapam, propylene oxide and allyl alcohol with a pipette, however, for methyl bromide a modified version of a Jiffy applicator was designed. Details and special procedures of the various experiments will be included in the presentation of results.

RESULTS FROM STUDIES OF FUMIGANT TOXICITY TO ANNUAL WEED SEED

The present investigation was directed mainly to an evaluation of the toxic effects of several soil fumigants on dormant and non-dormant seed of selected annual weed species. Seed were obtained either from stocks harvested and stored at 40°F, or from seed after-ripened under field conditions and washed from soil samples prior to exposure to soil fumigants. Additional variables were fumigants, fumigant concentrations, exposure times for seeds, temperatures during fumigation, and moisture content of weed seed. A further aspect of the study included a preliminary attempt to characterize weed seed viability and dormancy under field conditions from early fall, through winter and into late spring and early summer.

Toxicity of Soil Fumigants to Stored Weed Seed

Several seed lots of the species studied were available. Seed had been harvested from wild populations in 1963, and in prior years, and stored at 40°F. Both dormant and non-dormant seed were available generally, though not always, for Abutilon theophrasti, Setaria lutescens, Setaria faberi, and Polygonum pensylvanicum. Dormant seed of Abutilon theophrasti have a thick seed coat impermeable to water and to gas transfer according to Winter (53). Dormancy in seed of Polygonum pensylvanicum has been considered by LaCroix (26) and Justice (23) as maintained by germination inhibitors in the seed coat and by dormant embryos. Nieto (35) found seed of Setaria lutescens to have at least two mechanism responsible for dormancy, one maintained by lemma and palea and one by

the caryopsis. Seed lots were not identified as to year of harvest, but described as dormant or non-dormant. Petri dishes placed in fumigation chambers contained 50 seed on dry filter paper. Experiments were conducted at 68°F for the duration of exposure to the fumigant unless otherwise stated. Concentrations of the several fumigants were expressed in milligrams of fumigant per liter of air.

Fumigant concentration

Dry and moist seed were exposed to various concentrations of fumigants in fumigation chambers to determine the concentration which was toxic to seed, as measured by failure to germinate or loss of viability. Dry seed were those which had been air dried at maturity and stored at 40°F. Moist seed were obtained by placing seed on moist filter paper for a period of time to allow absorption of water such that the water content was above 15 percent. The moist condition implies that seed were wet on the surface, but does not reflect necessarily a change in the moisture percentage of the caryopsis or endosperm. Seed were exposed to fumigants for 24 hours in these studies.

Data presented in Table 1 illustrates the effect of concentration level of Vapam, propylene oxide, and allyl alcohol on the germination of non-dormant seed of Abutilon theophrasti, Setaria lutescens, and Setaria faberi. As concentrations in milligrams of fumigant per liter of air were increased, germination of weed seed was reduced. The importance of moisture content of seed was evident with all fumigant treatments. The effect was most pronounced on seed of Abutilon theophrasti fumigated with Vapam and propylene oxide. Allyl alcohol was the most toxic of the

Table 1. Germination percentages of non-dormant moist and dry intact weed seed fumigated with Vapam, propylene oxide, and allyl alcohol for 24 hours at 68°F

Concentration of fumigant mg/l	Species and seed condition					
	Abutilon theophrasti		Setaria lutescens		Setaria faberi	
	Moist	Dry	Moist	Dry	Moist	Dry
			Vapam			
1900	0	58	0	0	0	0
950	0	64	0	0	0	0
320	4	62	0	0	0	0
160	0	62	0	0	0	0
80	0	42	4	22	3	21
32	2	52	9	37	12	43
16	0	68	21	69	30	61
0	68		84		83	
			propylene oxide			
1400	1	54	0	0	0	0
700	1	62	0	0	0	0
230	0	56	0	0	0	0
116	4	57	1	0	0	1
58	9	57	4	18	1	30
11.6	5	62	76	89	53	82
2.3	64	60	83	88	85	83
1.2	60	66	86	86	85	82
0	60		84		83	
			allyl alcohol			
58	0	0	0	0	0	0
23	0	0	0	0	0	0
11.6	0	2	0	0	0	0
2.3	38	47	40	73	52	79
1.2	61	49	45	91	54	80
.23	34	40	41	83	60	76
0.0	65		86		77	

three fumigants tested on seed of Abutilon theophrasti followed by propylene oxide and Vapam in order of decreasing toxicity. Methyl bromide was toxic to both moist and dry seed of Setaria lutescens and Setaria faberi down to 235 mg/1, the lowest concentration which could be measured accurately with the applicator used. Exposure of Abutilon theophrasti seed to methyl bromide showed that moist seed were killed but dry seed were not injured with any of the concentrations used.

Effect of fumigation on dormant seed was determined either by the germination of caryopses with seed coats removed or by the growth of isolated embryos on nutrient medium. Results are summarized in Table 2. Dormant seed of Abutilon theophrasti were not killed by methyl bromide, Vapam, and propylene oxide even at the highest concentrations used; allyl alcohol, however, reduced germination at a concentration of 11.6 mg/1. Moist, non-dormant seed of Abutilon theophrasti were killed by exposure to methyl bromide, Vapam, and propylene oxide at concentrations much below those tolerated by dormant seed of Abutilon theophrasti. The toxic concentration of allyl alcohol was the same for both dormant and non-dormant seed. Concentrations of allyl alcohol and methyl bromide lethal to non-dormant seed of Setaria lutescens also killed dormant seed. Dormant seed of Setaria lutescens exposed to Vapam and propylene oxide appeared less susceptible than non-dormant seed. Later results suggested, however, that this was due probably to removal of the caryopses right after fumigation. An experiment in which germination was determined on caryopses removed after fumigation showed that both dormant and non-dormant seed of Setaria lutescens responded alike to each fumigant. Dormant seed of Polygonum pensylvanicum were killed with all levels of

Table 2. Germination percentages of embryos isolated from moist and dry dormant seed of *Abutilon theophrasti*, *Setaria lutescens* and *Polygonum pensylvanicum* following exposure to four fumigants for 24 hours at 68°F

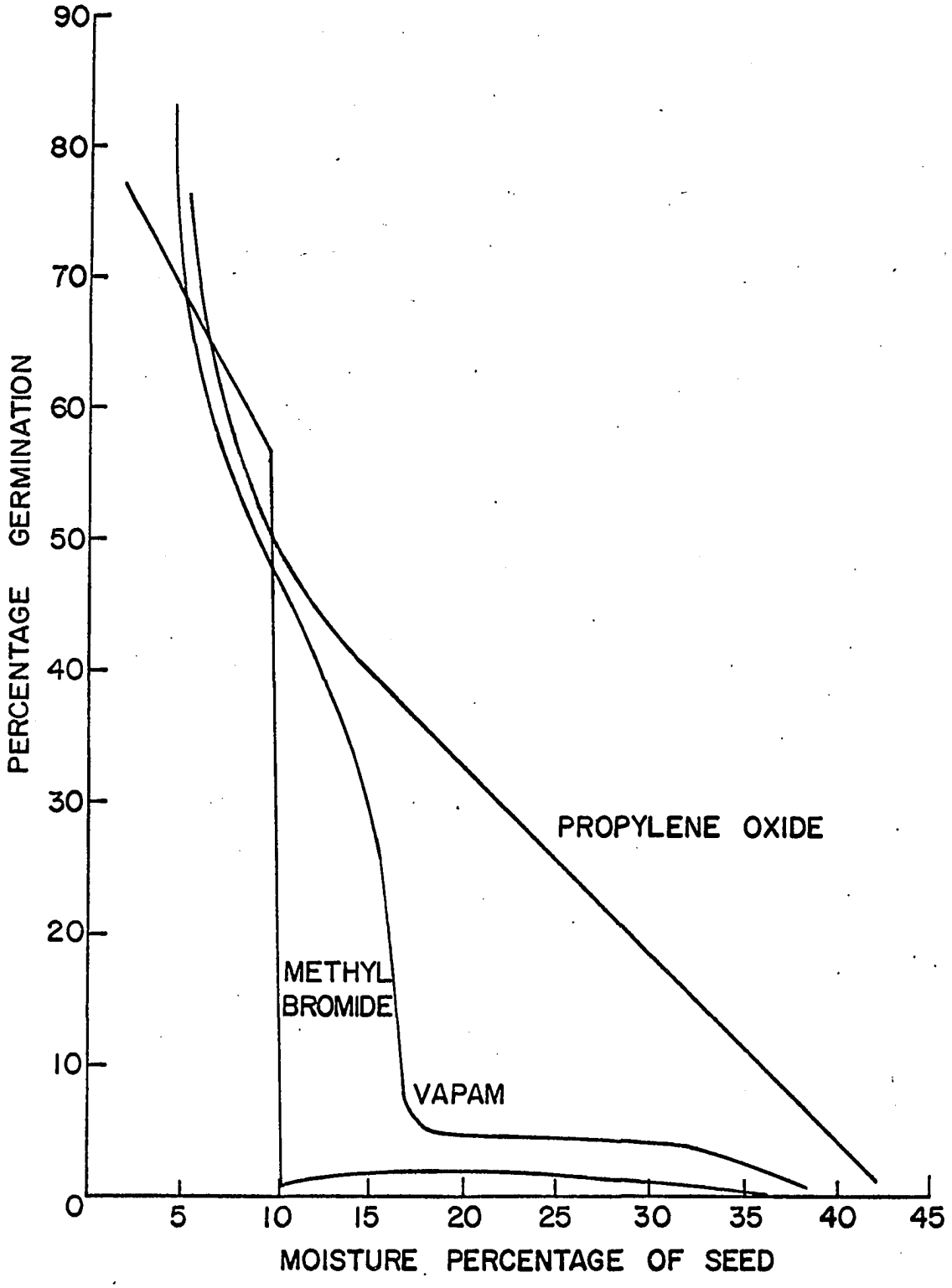
Species	Fumigant	Concentration of fumigant mg/l	Germination percentages embryo from seed	
			Moist	Dry
A. theophrasti	none	0	97	98
S. lutescens	none	0	80	80
P. pensylvanicum	none	0	75	80
A. theophrasti	Vapam	1900	96	98
A. theophrasti		950	97	99
A. theophrasti		320	100	98
S. lutescens		1900	75	70
S. lutescens		950	80	75
P. pensylvanicum		1900	70	65
P. pensylvanicum		950	10	60
A. theophrasti	allyl alcohol	116	0	0
A. theophrasti		11.6	0	38
A. theophrasti		2.3	94	92
A. theophrasti		1.2	92	96
A. theophrasti		.23	100	94
S. lutescens		11.6	0	0
S. lutescens		1.2	80	75
P. pensylvanicum		23	7	0
P. pensylvanicum		11.6	25	0
P. pensylvanicum		2.3	60	55
A. theophrasti	methyl bromide	2800	94	96
A. theophrasti		470	98	90
S. lutescens		235	0	0
P. pensylvanicum		235	0	0
A. theophrasti	propylene oxide	1400	88	98
A. theophrasti		230	98	90
S. lutescens		700	0	0
S. lutescens		46	40	85
P. pensylvanicum		700	0	0
P. pensylvanicum		230	0	0
P. pensylvanicum		116	0	0
P. pensylvanicum		11.6	0	0

methyl bromide, propylene oxide, and with the higher concentrations of allyl alcohol, but were not affected by fumigation with Vapam. No seed of non-dormant Polygonum pensylvanicum were available for testing.

Seed moisture

Moisture content of seed during fumigation has been reported to have a pronounced effect on susceptibility to fumigants. Greater moisture content enhances fumigant absorption, chemical reaction and movement of the fumigant into the seed. Two methods were employed to obtain seed in various moisture conditions; in one seed were stored at various levels of relative humidity and in the other seed were placed on moist filter paper for varying periods of time. Moisture content was determined by drying at 104° C for 48 hours. The technique of using relative humidity chambers was of limited value because significant changes in moisture content were obtained only after long periods of time at 100 percent relative humidity. These lengthy storage times permitted growth of fungi and resulted in very uneven absorption of water by individual seeds. Placement of seed on moist filter paper as a means of increasing moisture content was of limited value for Setaria lutescens and Setaria faberi but proved most effective for seed of Abutilon theophrasti. In this species water was absorbed fairly uniformly by seed coat, endosperm, and embryo. For this reason seed of Abutilon theophrasti were used in subsequent studies of the relationship of seed moisture content to observed fumigant toxicity. Results observed with seed of Abutilon theophrasti exposed to the several fumigants, summarized in Figure 1, illustrated the relationships between moisture content of seed and susceptibility to

Figure 1. Germination of seed of Abutilon theophrasti following exposure to 950 mg/1 Vapam, 700 mg/1 propylene oxide, or 470 mg/1 methyl bromide at various seed moistures for 24 hours at 68° F


90
80
70
60
50
40
30
20
10
0
PERCENTAGE GERMINATION
PROPYLENE OXIDE
METHYL
BROMIDE
VAPAM
5
10
15
20
25
30
35
40
45
MOISTURE PERCENTAGE OF SEED

fumigation. Each fumigant demonstrated a different pattern of toxicity as seed moisture varied. Allyl alcohol was extremely toxic and killed Abutilon theophrasti seed at moisture percentages down to five percent, the air dry condition. Effectiveness of propylene oxide increased gradually over the range of 5 to 42 percent moisture in the seed. Vapam killed seed of Abutilon theophrasti at moisture levels of 17 percent and above and gradually decreased in effectiveness as moisture percentage decreased to around five percent. Vapam toxicity was increased over a range of 13 percent, while that of propylene oxide extended over a range of 36 percent. Methyl bromide showed a gradual increase in toxicity between five and nine percent moisture and than a very sharp increase between nine and ten percent where almost 100 percent kill resulted. Critical moisture content or that moisture percentage at which fumigation had a markedly different effect on moist and dry seed, for the four fumigants on seed of Abutilon theophrasti was approximately 9.5 percent for methyl bromide, none for allyl alcohol and over a range of from ten percent and up for Vapam and propylene oxide.

Setaria lutescens and Setaria faberi seed sorbed water so rapidly that in 1 hour the moisture content was high enough to give large differences in germination following fumigation of moist and dry seed. It was difficult in such a short time to obtain an accurate measure of the moisture content of the seed sample. Uniform drying of seed prior to weighing with the small amount of water involved and uneven water sorption were the main difficulties encountered. The most reliable estimate of a critical moisture content was 9 to 18 percent depending upon the fumigant used.

Importance of water vapor in the soil atmosphere during fumigation was investigated by exposing dry seed in different conditions of relative humidity. Seed were fumigated for 24 hours in closed containers with relative humidities of 25, 50, 87, and 100 percent obtained with solutions of glycerol and water prepared according to Washburn (51, p. 291). Germination percentages of dry seed of Abutilon theophrasti, Setaria lutescens, and Setaria faberi fumigated at different relative humidities are summarized in Table 3. Concentrations of the fumigants used were those known to be toxic to moist seed and not to dry seed. Seed of Setaria lutescens fumigated with Vapam showed greater germination at 25 and 50 percent relative humidities than at 87 and 100 percent where almost complete kill occurred. Seed of Abutilon theophrasti and Setaria faberi fumigated with Vapam did not show a response to the different relative humidities. Toxicity of allyl alcohol, methyl bromide, and propylene oxide to seed of Abutilon theophrasti, Setaria lutescens, and Setaria faberi was not altered by different relative humidities as measured by germination of these weed species. Moisture content of seed after treatment was not determined and the effect of a wetting or drying action may be a factor in the observed results. The increase in germination observed at 25 percent relative humidity for Abutilon theophrasti fumigated with Vapam or propylene oxide may be the result of a drying action, which fractured the seed coat in the chalazal region and terminated dormancy.

Table 3. Germination percentages of seed of Abutilon theophrasti, Setaria lutescens, and Setaria faberi exposed for 24 hours at 68°F to four fumigants under various conditions of relative humidity

Fumigant	Concentration of fumigant mg/l	Weed species	Percent relative humidity 25	50	87	100
Allyl alcohol	11.6	A. theophrasti	0	0	0	0
	2.3	S. lutescens	0	0	0	0
	2.3	S. faberi	0	0	0	0
Methyl bromide	470	A. theophrasti	56	54	58	59
	235	S. lutescens	0	0	0	0
	235	S. faberi	0	0	0	0
Propylene oxide	700	A. theophrasti	77	71	73	72
	58	S. lutescens	2	0	0	9
	58	S. faberi	0	0	0	2
Vapam	950	A. theophrasti	76	73	68	66
	32	S. lutescens	40	24	18	2
	32	S. faberi	0	0	5	6

Exposure time

Effect of length of exposure of seed to fumigants was investigated using moist and dry seed of Abutilon theophrasti, Setaria lutescens, and Setaria faberi. Seed coats were removed to assess their effect in modifying the length of exposure required for maximum fumigant toxicity. Exposure periods to the various fumigants were chosen on the basis of previously determined toxicity patterns.

Germination percentages following different exposure periods to Vapam are presented in Table 4. Moist or dry Setaria lutescens seed

Table 4. Germination percentages for moist and dry seed of four species exposed for various periods to 950 mg/l Vapam at 68°F

Species	Seed condition	Exposure time in minutes							
		0	15	30	60	180	360	720	1440
Setaria lutescens	dry	30	28	20	20	18	4	1	0
	moist	30	32	18	6	10	1	0	0
Setaria faberi	dry	71	72	74	74	32	4	2	0
	moist	71	80	78	76	12	7	0	0
Abutilon theophrasti	moist	86	88	80	76	20	0	0	0
	Seed coat removed								
	dry	100	100	100	100	100			
	moist	100	90	90	30	0			
Polygonum pensylvanicum									
	Seed coat removed								
	dry	80	80	70	40	30			
	moist	80	50	30	30	10			

showed a similar decrease in germination as exposure time to Vapam was increased. Moist or dry seed of Setaria faberi were not injured with a 1 hour exposure but showed a sharp decrease in germination with exposures of 3 hours. Vapam did not kill intact seed of Polygonum pensylvanicum with exposures of up to 7 days. When seed coats of Polygonum pensylvanicum were removed a reduction in germination after a 30 minute exposure to Vapam was observed, but only with moist seed. Dry, non-dormant

Abutilon theophrasti seed were not killed after 7 days exposure to Vapam, but moist seed were killed with exposures of 3 hours. With seed coats removed, moist seed of Abutilon theophrasti showed a progressive decrease in germination and were killed after 3 hours exposure, but dry seed with seed coats removed showed no effect of fumigation after 3 hours. Dormant seed of Abutilon theophrasti exposed to Vapam for 14 days were not killed as measured by germination with seed coats removed.

Toxic levels of methyl bromide produced a very rapid killing action. Fumigation of moist intact seed of Setaria lutescens and Setaria faberi and Abutilon theophrasti and Polygonum pensylvanicum with seed coats removed showed almost complete kill in 15 minutes. Germination of dry seed of Polygonum pensylvanicum and Abutilon theophrasti exposed, with seed coats removed, was not affected after exposures of 3 hours to methyl bromide. The variation in results obtained with seed of Setaria lutescens fumigated for 15, 30, and 60 minutes is not explained readily from the data obtained, however, the same patterns occurred in three experiments with two replications in each. Table 5 summarizes data on length of exposure to methyl bromide.

Germination data for seed fumigated with allyl alcohol are presented in Table 6. All seed were killed after exposures of 30 minutes to allyl alcohol. Dormant seed of Abutilon theophrasti and Polygonum pensylvanicum exposed to allyl alcohol for 1 hour or less showed limited germination but all seedlings were necrotic. With seed coats removed, moist Abutilon theophrasti seed were killed after exposure of 5 minutes and dry seed were killed after exposure of 10 minutes to allyl alcohol. The same toxicity patterns occurred with moist and dry intact Abutilon theophrasti

Table 5. Germination percentages for moist and dry seed of four species exposed for various periods to 470 mg/l methyl bromide at 68°F

Species	Seed condition	Exposure time in minutes 0	15	30	60	180
Setaria lutescens	dry	30	45	33	46	24
	moist	30	9	4	14	0
Setaria faberi	dry	82	80	82	80	23
	moist	82	12	4	0	0
Abutilon theophrasti	moist	84	80	68	44	2
	Seed coat removed					
	dry	100	100	100	100	100
	moist	100	0	0	0	0
Polygonum pensylvanicum	Seed coat removed					
	dry	70	70	70	35	60
	moist	70	20	10	0	0

seed. Setaria lutescens and Setaria faberi seed were less susceptible and were not killed with exposures of less than 15 minutes. Moist seed were not as susceptible as dry.

Germination data for seed exposed to propylene oxide for short periods reflected the same general pattern of toxicity observed previously with this fumigant; a progressive increase in toxicity with increased exposure times. These data are summarized in Table 7. When seed coats

Table 6. Germination percentages for moist and dry seed of four species exposed for various periods to 115 mg/l allyl alcohol at 68°F

Species	Seed condition	Exposure time in minutes 0	5	10	15	30	60	180
Setaria lutescens	dry	45	44	37	36	0	0	
	moist	45	50	43	38	0	0	
Setaria faberi	dry	78	69	0	0	0	0	0
	moist	78	82	74	64	0	0	0
Abutilon theophrasti	dry	86	92	8	0	0	0	0
	moist	86	4	4	0	0	0	0
	dormant[a]	100	100	24	60	0	20	
	Seed coats removed							
	dry	100	100	0	0	0	0	
	moist	100	0	0	0	0	0	
Polygonum pensylvanicum	dormant[a]	70	40	30	0	20	0	
	Seed coats removed							
	dry	70	30	0	0	0	0	
	moist	70	0	0	0	0	0	

[a]Germination of excised embryo.

were removed from moist seed, Polygonum pensylvanicum and Abutilon theophrasti showed a rapid decrease in germination after exposures of 15 minutes. Germination of dry Setaria faberi seed was quite variable following exposure to propylene oxide; the data presented are

Table 7. Germination percentages for moist and dry seed of four species exposed for various periods to 700 mg/l propylene oxide at 68°F

Species	Seed condition	Exposure time in minutes 0	15	30	60	180	360	730
Setaria lutescens	dry	40	40	38	36	44	26	0
	moist	40	12	12	0	0	0	0
Setaria faberi	dry	82	88	26	4	10	0	0
	moist	82	10	4	0	0	0	0
Abutilon theophrasti	moist	87	96	92	76	35	0	
	Seed coats removed							
	dry	100	100	100	100	100		
	moist	100	20	0	0	0		
Polygonum pensylvanicum								
	Seed coats removed							
	dry	75	70	90	50	40		
	moist	75	0	0	0	0		

representative of the results observed. Variability may have resulted from a stimulation of germination following sub-lethal exposure periods to toxic concentrations of the fumigant. These short periods varied between 30 and 60 minutes depending upon seed condition. Possible stimulation of seed germination with various exposure times was evident in the data summarized for the other fumigants.

Temperature

Effect of temperature on toxicity patterns of fumigants was measured by germination of non-dormant seed Setaria lutescens, Setaria faberi, Abutilon theophrasti and dormant seed of Abutilon theophrasti and Polygonum pensylvanicum after exposure of 24 hours at 50°, 68°, and 86°F. After release of the fumigant into the fumigation chamber, the chambers were stored at the appropriate temperature for the 24 hour exposure period. Initial tests run with high concentrations of fumigants showed no effect of temperature on fumigant toxicity over the range of 50° to 86°F. A second experiment was conducted using minimum toxic concentrations of the fumigant to moist seed at 68°F with the possibility that in a 18°F change the fumigant would become toxic to both moist and dry seed or neither. Table 8 summarizes the data on effect of temperature to fumigant toxicity. With lower concentrations there was no temperature effect on toxicity except for Setaria faberi seed fumigated with Vapam at 86°F and seed of Abutilon theophrasti and Setaria lutescens fumigated with propylene oxide at 86°F. Experiments also were conducted with length of exposure to fumigants as a variable, inclusive results were obtained. With exposure times of 2 hours to methyl bromide the effect of temperature on toxicity was demonstrated. Moist seed of Setaria lutescens and Setaria faberi were killed uniformly at 50°, 68° and 86°F, but dry seed revealed an increase in toxicity with an increase in temperature. Toxicity of methyl bromide to dry seed of Abutilon theophrasti was not affected by temperature and results with moist seed suggested an increase in fumigant toxicity with increase in temperature. Germination percentages with methyl bromide toxicity response to

Table 8. Germination percentages of moist and dry seed of *Abutilon theophrasti*, *Setaria lutescens*, *Setaria faberi*, and *Polygonum pensylvanicum* exposed for 24 hours to three fumigants at three temperatures

Fumigant	Concn. mg/l	Species	Temperature in degrees F: 50° moist	50° dry	68° moist	68° dry	86° moist	86° dry
None	0	*A. theophrasti*	63		68		58	
	0	*A. theophrasti* (dormant)	100		100		100	
	0	*S. lutescens*	68		68		68	
	0	*S. faberi*	89		91		88	
	0	*P. pensylvanicum*	75		75		75	
Vapam	950	*A. theophrasti*	0	60	0	64	1	43
	16	*A. theophrasti*	62	57	55	58	48	61
	950	*A. theophrasti* (dormant)	-	-	97	99	100	84
	950	*S. lutescens*	0	0	0	0	0	0
	16	*S. lutescens*	15	37	14	21	27	15
	950	*S. faberi*	0	0	0	0	0	0
	16	*S. faberi*	27	34	9	40	13	14
	950	*P. pensylvanicum*	-	-	10	60	0	0
Propylene oxide	700	*A. theophrasti*	4	76	1	62	0	46
	11.6	*A. theophrasti*	63	50	58	63	53	47
	700	*A. theophrasti* (dormant)	-	-	88	98	100	72
	700	*S. lutescens*	0	2	0	0	0	0
	11.6	*S. lutescens*	80	88	89	80	54	84
	700	*S. faberi*	0	0	0	0	0	0
	11.6	*S. faberi*	76	63	76	72	77	69
	700	*P. pensylvanicum*	-	-	0	0	0	0
Allyl alcohol	23	*A. theophrasti*	0	0	0	0	0	0
	1.2	*A. theophrasti*	67	50	53	49	55	53
	23	*A. theophrasti* (dormant)	0	0	0	0	0	0
	23	*S. lutescens*	0	0	0	0	0	0
	1.2	*S. lutescens*	84	69	61	78	74	68
	23	*S. faberi*	0	0	0	0	0	0
	1.2	*S. faberi*	76	63	76	72	77	69
	23	*P. pensylvanicum*	0	0	0	0	0	0

Table 9. Germination percentages of moist and dry seed of Abutilon theophrasti, Setaria lutescens, and Setaria faberi exposed for 2 hours to 470 mg/1 methyl bromide at three temperatures

Species	Temperature in degrees F					
	50°		68°		86°	
	moist	dry	moist	dry	moist	dry
A. theophrasti	54	68	24	67	43	62
S. lutescens	0	54	0	49	0	31
S. faberi	0	69	0	60	0	38

temperature are summarized in Table 9.

Toxic action of Vapam

In all experiments intact seed of Setaria lutescens did not germinate following exposure to Vapam. However, the excised embryos or the intact caryopses from these seed germinated normally. Experiments then were designed to investigate the physical condition of the seed when the embryo was killed by fumigation with Vapam. Moist or dry seed of Setaria lutescens were exposed for 24 hours to a medium concentration of Vapam (950 mg/1). Immediately after fumigation, experimental seed lots were handled as follows; lemma and palea removed from caryopses, lemma and palea were clipped at one end and left on the caryopses, and one lot was left intact. Germination percentages for each of the three experimental lots were determined as follows: (1) immediately after fumigation, (2) after a period of dry storage in the laboratory, and (3) after storage under conditions for optimum germination of untreated

Table 10. Germination percentages of seed of Setaria lutescens exposed to 950 mg/l Vapam for 24 hours, followed by storage for periods of time

Seed manipulation and storage	Germination percentages for days following fumigation						
	0	4	7	10	13	25	50
Intact seed, stored dry	5	0		0			
Lemma and palea clipped, stored dry	7	0		0			
Lemma and palea removed after exposure, stored dry	76	65	72	72	60	75	78
Lemma and palea removed at time of germination test from intact seed, stored dry		100	88	92	20	50	65
Lemma and palea removed at time of germination test from intact seed, stored under germinating conditions		35	5	0		0	0
Lemma and palea removed at time of germination test from seed with lemma and palea clipped, stored dry		95	90	85	75	75	60
Lemma and palea removed at time of germination test from seed with lemma and palea clipped, stored under germinating conditions		50	25	35		0	0

seed. Results are summarized in Table 10. Immediately following fumigation, germination percentages of seed from lots left intact or with lemma and palea clipped were low, but in lots with lemma and palea removed no decrease in germination was observed. Four days after fumigation experimental lots, left intact or with lemma and palea clipped, and stored under germinating conditions had a high percentage of dead

embryos. When stored dry, however, caryopses of intact or clipped seed showed high germination percentages. When lemma and palea were removed immediately after fumigation and the caryopses stored dry no reduction in germination was observed after 50 days. Caryopses of intact and clipped seed stored dry showed the same response as caryopses alone. Intact and clipped seed stored under germinating conditions showed decrease in germinability of caryopses for about 25 days when all were dead. Viability of seed was determined by excision and culture of embryos and was supplemented with tetrazolium tests. Further experiments were conducted to determine the susceptibility of the caryopses alone and embryos alone when exposed to fumigation. The embryo was killed readily by fumigation with Vapam but the caryopsis showed only a partial reduction in germination when fumigated with Vapam under dry conditions.

After-ripening of *Polygonum pensylvanicum* and *Setaria lutescens* Seed in the Field

Seed produced by *Setaria lutescens* and *Polygonum pensylvanicum* are dormant generally when matured. This dormancy may be maintained for extended periods when the seed is stored dry, at cool temperatures. Nieto (35) reported that low temperature stratification in the laboratory usually terminated dormancy in *Setaria lutescens*. Moderate success with similar technique with *Polygonum pensylvanicum* has been reported by Justice (23). After-ripening under natural conditions in the field during fall and winter normally results in germination and seedling development the following spring.

This study of after-ripening patterns of seed of *Setaria lutescens*

and Polygonum pensylvanicum presents a preliminary assessment of weed seed dormancy and germinability under field conditions during the period from early fall to early summer of the next season. Depth of seed placement in the soil, soil temperature, and soil moisture investigated as possible factors bearing on termination of dormancy in these species. Relationships of these factors to viability, germination and seedling emergence in the field, and susceptibility to fumigation during the after-ripening process also were explored. Incomplete germination of intact seed, which germinated freely with seed coats removed, led to studies of the possible role of seed coats as physical barriers to germination or as sites of natural germination inhibitors.

Mature seed of Setaria lutescens and Polygonum pensylvanicum from 1963 seed lots were dormant at time of placement in soil. Isolated embryos cultured on suitable medium germinated 80 and 100 percent, respectively indicating no embryo dormancy. Caryopses of Setaria lutescens did not germinate under laboratory conditions. Dormancy in Polygonum pensylvanicum appeared to be conditioned by the seed coat since 80 percent germination occurred when seed coats were removed. Data are summarized in Table 11 for the germination of the seed lots prior to placement in field soil in November, 1963.

Table 11. Germination percentages and moisture content of 1963 seed of Setaria lutescens and Polygonum pensylvanicum prior to burial at various depths in the field

Weed species	Moisture percentage of seed	Germination percentages		
		Intact seed	Isolated embryos	Seed coats removed
Setaria lutescens	9.4	0	80	0
Polygonum pensylvanicum	9.2	0	100	80

Dormancy studies

Freshly harvested, 1963 seed of Setaria lutescens and Polygonum pensylvanicum were extremely dormant. Tests of these seed lots indicated that the seed coats or the lemma and palea inhibited germination. Data, presented in Table 11, illustrate the effect of the seed coat removal on germination of Setaria lutescens and Polygonum pensylvanicum. The effect of the seed coat in preventing germination of intact seed of Setaria lutescens and Polygonum pensylvanicum suggested a possible role of inhibitors in the seed coat. This was investigated by leaching experiments, using cold water in the closed recirculating system described by LaCroix (26).

Germination percentages of Setaria lutescens and Polygonum pensylvanicum after leaching are summarized in Table 12. Leaching in cold water for 48 hours did not increase germination of intact seed of either species. For seed of Setaria lutescens, with lemma and palea clipped before leaching, with lemma and palea removed before leaching, or with

Table 12. Germination percentages of seed of Setaria lutescens and Polygonum pensylvanicum following cold water leaching

Seed condition	Setaria lutescens Leaching time in hours		Polygonum pensylvanicum Leaching time in hours	
	0	48	0	48
Intact seed	0	0	0	0
Seed coat clipped	0	6	6	7
Seed coat removed after leaching	0	11	80	83
Lemma and palea removed before leaching	0	17		

lemma and palea removed after leaching only a slight increase in germination was observed, compared with intact seed. Seed of Polygonum pensylvanicum showed no increase in germination following cold water leaching. Dormancy of Polygonum pensylvanicum was associated with the seed coat since germination proceeded freely after the seed coat was removed. When the water leachate from 100 seed coats of Polygonum pensylvanicum was used as the moisture source for 100 seed with seed coats removed a reduction in germination was observed. The reduction was not large and the possibility of seed coats acting as a mechanical restriction to germination in Polygonum pensylvanicum remained. Experiments were conducted in which the seed coat was completely cut around the periphery with the endosperm remaining between the two halves and a second experiment was run in which the seed coat was cut around the periphery except for a very small portion. Results from both these

Table 13. Germination percentages of seed of Polygonum pensylvanicum water leachate used as moisture source or with seed coats removed or clipped

Seed condition	Germination percentage
Seed coat removed, no leachate added	64
Seed coat removed, leachate added	39
Seed coat cut completely around periphery	28
Seed coat cut most way around periphery	23

experiments, presented in Table 13, indicated that failure to germinate was not due to mechanical restriction.

Germination studies

Sampling techniques and germination methodology, described previously, were used in following the changes in germinability of Setaria lutescens and Polygonum pensylvanicum seed during after-ripening in the field. Table 14 summarizes the germination percentages of Setaria lutescens and Polygonum pensylvanicum seed sampled from the field during the winter of 1963-64. With seed of Setaria lutescens there was little change in the dormancy condition during the first 8 weeks of after-ripening in the soil. Germination of seed from the 6 inch depth in dry soil showed an indication of termination of dormancy after 8 weeks and by the thirteenth week seed from all depths, except the surface, were non-dormant. Dormancy was terminated in seed from dry soil earlier than in seed from wet soil and under both conditions the deeper the seed were

Table 14. Germination percentages of Setaria lutescens and Polygonum pensylvanicum after-ripened in wet or dry soil at various depths during the period November, 1963 to June, 1964

Weeks of after-ripening	Wet soil depth, inches				Dry soil depth, inches			
	0	1	3	6	0	1	3	6
Setaria lutescens								
0	0	0	0	0	0	0	0	0
3	1/4	0	1/4	1/4	1/4	1/2	1	1/2
5	1	1	2	5	1/4	1	2	3
8	1/4	1	5	2	1	5	2	9
10	1	3	6	5	1/4	5	14	22
13	9	16	32	45	1/4	68	79	80
16	6	8	28	36	0	-	76	76
18	7	18	38	46	9	67	86	82
20	13	24	51	54	-	41	90	94
22	29	52	69	78	34	56	78	89
24	62	43	77	87	-	86	85	89
31	16	2	11	38	-	-	-	-
Polygonum pensylvanicum								
0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
10	0	0	0	1/4	0	0	0	0
13	0	0	0	1/2	0	1/2	0	1/2
16	1	0	0	1/2	0	1/2	0	2
18	0	9	8	2	0	5	2	2
20	1/2	9	10	2	-	3	4	1
22	8	4	0	8	2	0	13	18
24	1	0	0	0	-	4	1	4
31	0	0	0	0	0	0	0	0

placed in the soil the faster dormancy was terminated. Maximum germination was observed by the thirteenth week with seed sampled at the 3 and 6 inch depths in dry soil while seed from the surface and 1 inch depths continued to show an increase in germination until the twenty-fourth week. Termination of dormancy in seed left on the soil surface was not observed until the twenty-second week with either dry or wet soil. In wet soil dormancy was terminated at an increasing rate at all depths until complete germination had occurred in the field. The decrease in observed germination after 31 weeks reflected the extent of seed germination which had occurred already in the field as well as the numbers of dormant seed remaining. Seed from dry soil germinated more readily after being placed in the germinators than seed from wet soil. Seed from dry soil reached maximum germination in 2 to 5 days and seed from wet soil did not reach maximum germination until 5 to 10 days. This difference was observed at several sampling dates; the results from a typical sampling date are summarized in Table 15.

Table 15. Germination percentages of Setaria lutescens sampled from various depths of wet and dry soil

Soil condition	Depth inches	Germination percentages after 2 days	5 days	10 days
Wet	0	2	6	6
	1	10	18	20
	3	8	24	48
	6	24	32	50
Dry	0	20	26	28
	1	67	67	67
	3	78	92	94
	6	76	96	96

Essentially no germination was observed from seed of Polygonum pensylvanicum sampled from the field 18 weeks after placement in the soil. Seed of Polygonum pensylvanicum sampled in April, 20 to 24 weeks after burial, showed little germination when seed were placed in the germinator, but observations of seedlings in the field showed that germination had begun the latter part of March. By mid-April seed buried at the 1 and 3 inch depths had germinated approximately 70 percent in wet soil in the field. Early germination of seed of Polygonum pensylvanicum in dry soil indicated a possible temperature effect resulting from the plastic covering. Any such effect was not evident with Setaria lutescens since seed from dry soil did not germinate until 6 weeks after Polygonum pensylvanicum and 2 weeks after Setaria lutescens seed germinated from wet soil. Germination of Setaria lutescens seed in the field was observed first on April 15, 22 weeks after burial and was completed essentially by June 15. Table 16 summarizes the germination percentages of seed of Setaria lutescens and Polygonum pensylvanicum observed in the field. The large differences in germination percentages of seed of both species observed with wet and dry soil may have been due to a specific moisture requirement for germination. This possibility was supported by observed increased germination in dry soil during mid-May when the plastic cover was removed from the plots. Also, the increased germination might have been an effect of a moisture requirement for after-ripening, but this was not supported by germination data of Setaria lutescens seed obtained in other experiments.

The increased germination of Polygonum pensylvanicum which was observed in the field as compared with that obtained in the laboratory,

Table 16. Germination percentages of Setaria lutescens and Polygonum pensylvanicum observed from various depths in the field during the spring of 1964

Date	Wet soil depth, inches				Dry soil depth, inches			
	0	1	3	6	0	1	3	6
Setaria lutescens								
March 19	0	0	0	0	0	0	0	0
April 2	0	0	0	0	0	0	0	0
April 15	0	1	3	0	0	0	0	0
April 30	15	20	25	20	-	0	10	15
May 7	20	20	25	20	-	5	15	15
May 14	20	20	30	30	-	5	20	25
June 17	20	45	60	70				
Polygonum pensylvanicum								
March 19	0	0	0	0	0	15[a]	0	0
April 2	0	0	0	0	-	5	5	0
April 15	1	90	70	10	0	5	8	5
April 30	15	70	50	15	-	5	10	5
May 7	15	75	50	15	10	10	15	5
May 14	20	85	55	20	-	-	30	10
June 17	20	90	60	20				

[a]Due to one replication.

suggested either that termination of dormancy in the field was followed by immediate germination or that conditions in the laboratory were unsuitable for optimum germination. This latter possibility was checked by germinating samples under alternating temperatures of 68°-86°F and 59°-86°F for 16 hours at the lower temperature and 8 hours at the higher temperature. Under these conditions no increase in germination was observed.

Germination and soil moisture

The greater germination, observed in wet soil compared to germination in dry soil, prompted studies to determine soil moisture percentages required for weed seed germination. No non-dormant seed of Polygonum pensylvanicum were available, therefore non-dormant seed of Abutilon theophrasti and Setaria lutescens were used in this study.

Germination of weed seed in soil at moisture percentages of field capacity and below was investigated with Clarion loam soil. At field capacity this soil contained 21 percent moisture and at the permanent wilting point the moisture percentage was approximately 9.5 percent. Fifty grams of soil, dried 48 hours at 104°C, the appropriate amount of water for soil moisture percentages of 20, 16, 12, and 8 percent, and 25 seed of Abutilon theophrasti and Setaria lutescens were mixed thoroughly and sealed in a petri dish. Results of germination tests after 10 days are summarized in Table 17. Seed of Abutilon theophrasti germinated with 20 and 16 percent soil moisture, while seed of Setaria lutescens germinated only with 20 percent soil moisture. Seed of Abutilon theophrasti at 12 and 8 percent soil moisture imbibed water but did not germinate.

Table 17. Germination percentages of seed of Abutilon theophrasti and Setaria lutescens in four soil moistures

Soil moisture percentages after		Germination percentages after 10 days	
0 days	10 days	A. theophrasti	S. lutescens
20	16.4	78	10
16	13.3	70	0
12	10.1	0	0
8	6.8	0	0
moist filter paper		84	45

Viability

Viability tests as determined by embryo isolation and culture on suitable medium showed that, with both species studied, the embryos did not become dormant when left in the soil in the field. After the first month, Polygonum pensylvanicum showed a definite increase in seedling vigor and dormancy of Setaria lutescens seed conditioned by the caryopsis had been terminated. These results permitted viability tests to be run on seed with the seed coats removed and embryo isolation was used only as a occasional check. Viability data are summarized in Table 18.

Seedling emergence

Observations of seedling emergence from various depths in the field showed that Polygonum pensylvanicum emergence began the second week in April and emergence of Setaria lutescens started one week later. Setaria lutescens reached maximum emergence by mid-May while Polygonum

Table 18. Germination percentages of excised embryos of Setaria lutescens Polygonum pensylvanicum after-ripened in wet or dry soil at various depths during the period November, 1963 to May, 1964

Weeks of after-ripening	Wet soil depth, inches				Dry soil depth, inches			
	0	1	3	6	0	1	3	6
Setaria lutescens								
0	80	80	80	80	80	80	80	80
3	70	95	75	85	70	65	90	85
5	95	100	90	95	95	90	95	95
8	70	60	50	70	30	70	90	100
10	30	90	60	70	40	80	80	80
13	70	65	50	70	30	-	80	90
16	60	50	80	90	30	-	90	90
18	80	80	100	90	50	-	90	90
20	80	80	90	90	-	-	90	100
22	50	50	60	20	50	-	70	70
24	100	80	60	90	-	-	90	90
Polygonum pensylvanicum								
0	100	100	100	100	100	100	100	100
3	80	85	70	65	80	80	80	70
5	100	-	-	100	100	30	90	100
8	50	40	70	70	50	70	80	100
10	55	60	50	50	30	70	70	70
13	75	80	80	90	35	90	70	95
16	90	90	70	80	90	-	60	80
18	20	60	70	50	70	-	100	100
20	40	80	60	60	-	60	70	100
22	60	40	90	60	80	-	100	70
24	70	60	90	90	-	-	70	100

pensylvanicum emergence continued high until mid-June. Seedling emergence followed essentially the germination patterns observed in the field, namely, greater emergence from wet soil than dry soil. Again, this pattern was the opposite of that observed for termination of dormancy with seed of Setaria lutescens where seed in dry soil showed termination of dormancy earlier and to a greater extent than seed after-ripened in wet soil. The percentage of seedlings which emerged from the 3 and 6 inch depths was very low. Seed sampled from these depths showed epicotyl development that often extended nearly to the soil surface. This was true particularly with Setaria lutescens and Polygonum pensylvanicum from the 6 inch depth. Table 19 summarizes emergence data for Setaria lutescens and Polygonum pensylvanicum in the field.

Soil temperature

Soil temperature data were obtained from the weather station located 1 mile southwest of the experimental area. The number of days, when the maximum and minimum soil temperature at the 1 and 8 inch depths was 32°F or within certain limits of this temperature is shown in Table 20. Time period included was December 1 to February 14, when dormancy was terminated and maximum germination was obtained with seed of Setaria lutescens excavated from the 6 inch depth. Maximum temperature at the 1 inch depth was 32°F, plus or minus 2°F, for 52 days and the minimum temperature was within this range for 25 days. At the 8 inch depth the maximum and minimum temperatures were in the 30° to 34°F range for 56 and 49 days, respectively. At the 8 inch depth the maximum and minimum temperatures were nearly the same and usually the day the maximum temperature was

Table 19. Percent emergence of Setaria lutescens and Polygonum pensylvanicum from various depths in the field in wet or dry soil in the field during the spring of 1964

Date	Wet soil depth, inches				Dry soil depth, inches			
	0	1	3	6	0	1	3	6
Setaria lutescens								
April 15	0	0	0	0	0	0	0	0
April 30	7	8	5	0	0	0	T[a]	0
May 7	10	10	8	0	2	2	2	0
May 14	15	10	10	T	-	10	10	3
June 17	15	10	10	T	-	-	-	-
Polygonum pensylvanicum								
April 15	0	0	0	0	2	1	1	0
April 30	3	30	15	0	-	2	1	0
May 7	5	70	20	0	15	2	10	0
May 14	5	80	15	2	-	3	12	2
June 17	20	90	40	3	-	-	-	-

[a]Less than one percent emergence.

Table 20. Number of days maximum and minimum soil temperatures from the 1 and 8 inch depths were within stated limits. Period included December 1, 1963 to February 14, 1964

Degrees fluctuation from 32°F	1 inch depth		8 inch depth	
	Max.	Min.	Max.	Min.
0	13	5	35	24
1	29	11	13	16
2	10	9	8	9
3-5	16	27	16	26
greater than 5	8	24	4	1

within 2°F of 32°F the minimum temperature was also. This pattern did not occur at the 1 inch depth where much greater fluctuation of temperature was observed. Setaria lutescens dormancy was terminated first at the 6 inch depth. Soil temperatures at the 8 inch depth during the after-ripening period provided the conditions of low temperature stratification. At the 1 inch depth soil temperatures did not provide such conditions, and seed of Setaria lutescens from 1 inch depth did not germinate as well.

Precipitation totaled 3.7 inches in the first 20 weeks of after-ripening. Over 1 inch occurred immediately after burial of seed and 2.3 inches had accumulated by the time dormancy was terminated in Setaria lutescens seed. However, termination of dormancy conditioned by the caryopsis occurred soon after placement in the soil. Rainfall data are summarized in Figure 2.

Figure 2. Rainfall accumulation for late fall and early winter of 1963 and 1964 at Ames, Iowa

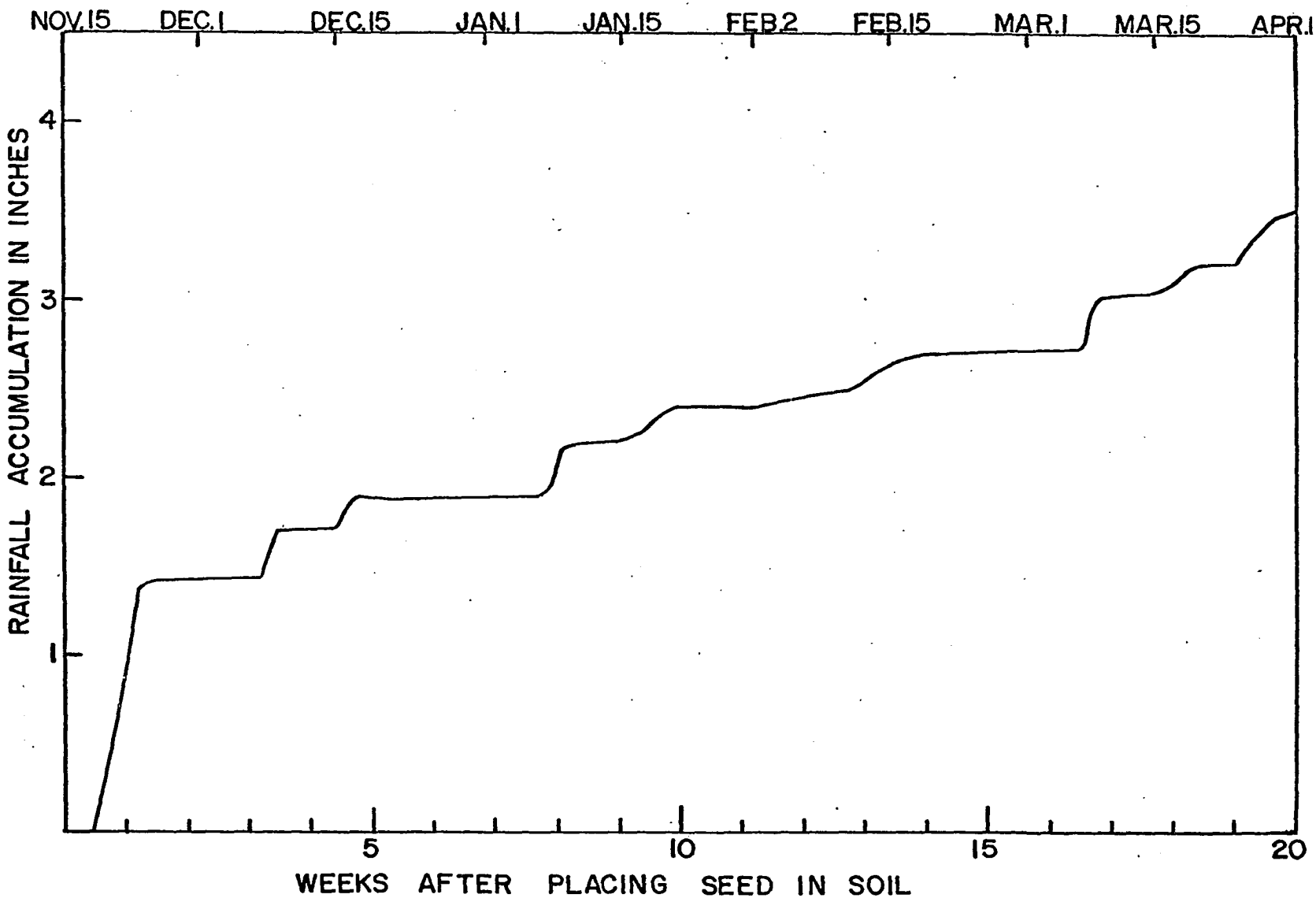

NOV.15
DEC.1
DEC.15
JAN.1
JAN.15
FEB.2
FEB.15
MAR.1
MAR.15
APR.1
RAINFALL ACCUMULATION IN INCHES
1
2
3
4
5
10
15
20
WEEKS AFTER PLACING SEED IN SOIL

Table 21. Moisture percentages of seed of Setaria lutescens and Polygonum pensylvanicum after-ripened in wet or dry soil at 2 depths during the period November, 1963 to May, 1964

Weeks of after-ripening	Wet soil depth, inches		Dry soil depth, inches	
	0	6	0	6
Setaria lutescens				
0	9.4	9.4	9.4	9.4
5	7.0	7.4	10	10.6
8	8.3	8.0	9.0	8.9
14	7.7	7.6	8.5	8.0
22	7.6	9.6	8.7	6.9
Polygonum pensylvanicum				
0	9.2	9.2	9.2	9.2
5	7.5	7.7	9.2	9.6
8	8.2	8.5	8.6	8.8
14	5.6	9.1	6.9	7.5
18	6.2	8.4	5.8	6.4

Moisture content of seed

Data for moisture content of the buried seed during the after-ripening period are summarized in Table 21. Moisture content of seed was generally higher at the 6 inch depth than with surface placement. This was true especially for seed of Polygonum pensylvanicum. Wet or dry soil had little or no effect on moisture content of seeds. Moisture content in seed of both species decreased as after-ripening progressed,

indicating a drying of the seed as dormancy was terminated. Fluctuation in moisture content of seed was due probably to sampling error and did not reflect a significant change in water content of the seed.

Water uptake studies

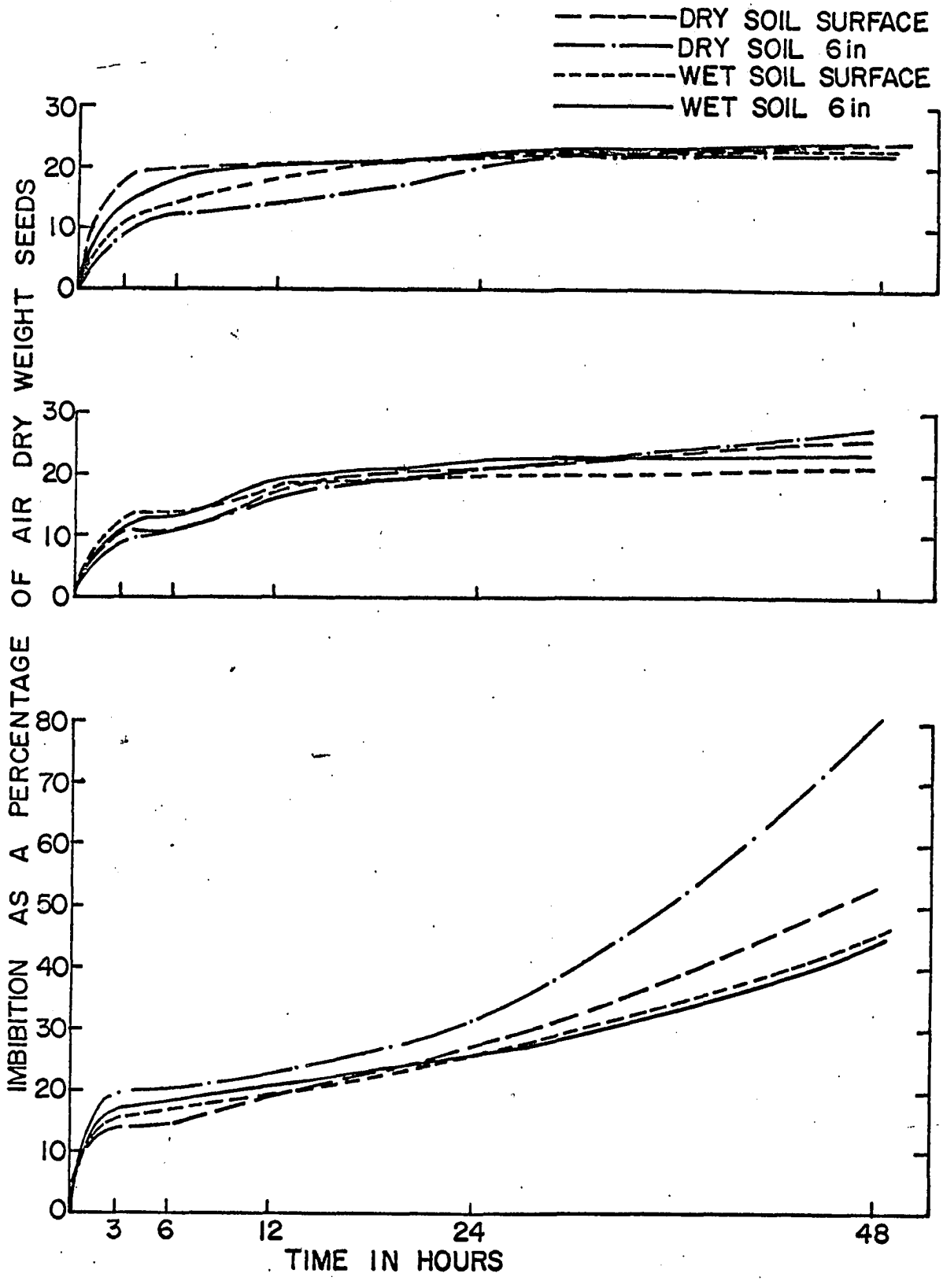
Absorption of water precedes germination, but germination may not always follow water uptake. Water uptake at 77°F by seed of Setaria lutescens and Polygonum pensylvanicum was determined for seed removed from the surface and excavated from 6 inch depths. Two samples of 50 seed from each depth and soil condition were weighed and placed in petri dishes containing filter paper and water. Seed samples were removed and weighed after 3, 6, 12, 24, and 48 hour exposures to water. The imbibed seed were surface dried before weighing. The results obtained with Setaria lutescens seed are summarized in Figures 3, 4, and 5.

Data for seed sampled January 8, 8 weeks after burial, are shown in Figure 3. No differences in water uptake were evident during the first 48 hours. Germination of these samples was low. Water uptake by seed sampled February 14, 13 weeks after burial, are shown in Figure 4 and again reflect no difference in water uptake during the first 48 hours among depths and soil conditions. On this sampling date, germination of seed from the surface of dry or wet soil was less than 1 percent and 9 percent respectively, whereas seed from the dry or wet soil at the 6 inch depth showed 80 and 45 percent germination, respectively, after 10 days. Water uptake of seed from the 6 inch depths, sampled February 14, did not reflect the differences in germination compared with seed taken from the soil surface. Similarly, seed sampled January 8 and

Figure 3. Water absorption curves for intact seed of Setaria lutescens after-ripened eight weeks in wet or dry soil in the field at two depths

Figure 4. Water absorption curves for intact seed of Setaria lutescens after-ripened 13 weeks in wet or dry soil in the field at two depths

Figure 5. Water absorption curves for intact seed of Setaria lutescens after-ripened 22 weeks in wet or dry soil in the field at two depths


DRY SOIL SURFACE
DRY SOIL 6in
WET SOIL SURFACE
WET SOIL 6in
IMBIBITION AS A PERCENTAGE OF AIR DRY WEIGHT SEEDS
0
10
20
30
0
10
20
30
0
10
20
30
40
50
60
70
80
3
6
12
24
48
TIME IN HOURS

February 14 did not reflect the differences in germination that were observed at all depths for these two sampling dates. Figure 5 illustrates the greater water absorption with increased germination of seed sampled April 15, 22 weeks after burial. Germination tests also showed this faster rate of water absorption in that seed from dry soil had maximum germination in 2 to 5 days and seed from wet soil did not show maximum germination until 5 to ten days.

Water absorption data of seed of Polygonum pensylvanicum from the February 14 sampling date are summarized in Figure 6. The curves shown were typical for seed of Polygonum pensylvanicum during the after-ripening period. No differences occurred among wet or dry soils, or surface and 6 inch depth treatments. The 1963 Polygonum pensylvanicum seed stored at 40°F in the laboratory yielded almost an identical curve for water absorption. No change in water uptake by intact Polygonum pensylvanicum after-ripening in the field prompted a further experiment of water uptake by various dormant conditions in Polygonum pensylvanicum with seed coats removed. Seed used were the 1957 lot, which contained a dormant embryo; the 1963 seed lot stored at 40°F in the laboratory and 1963 seed from the field. The 1963 seed of Polygonum pensylvanicum contained non-dormant embryos. 1963 field seed showed a rapid and continuous rate of water absorption and 1963 laboratory seed had a curve similar to 1963 intact seed. 1957 seed showed absorption of the first 12 hours and then leveled off. These data are summarized in Figure 7.

Figure 6. Water absorption curves for intact seed of Polygonum pensylvanicum after-ripened 13 weeks in wet or dry soil in the field at two depths

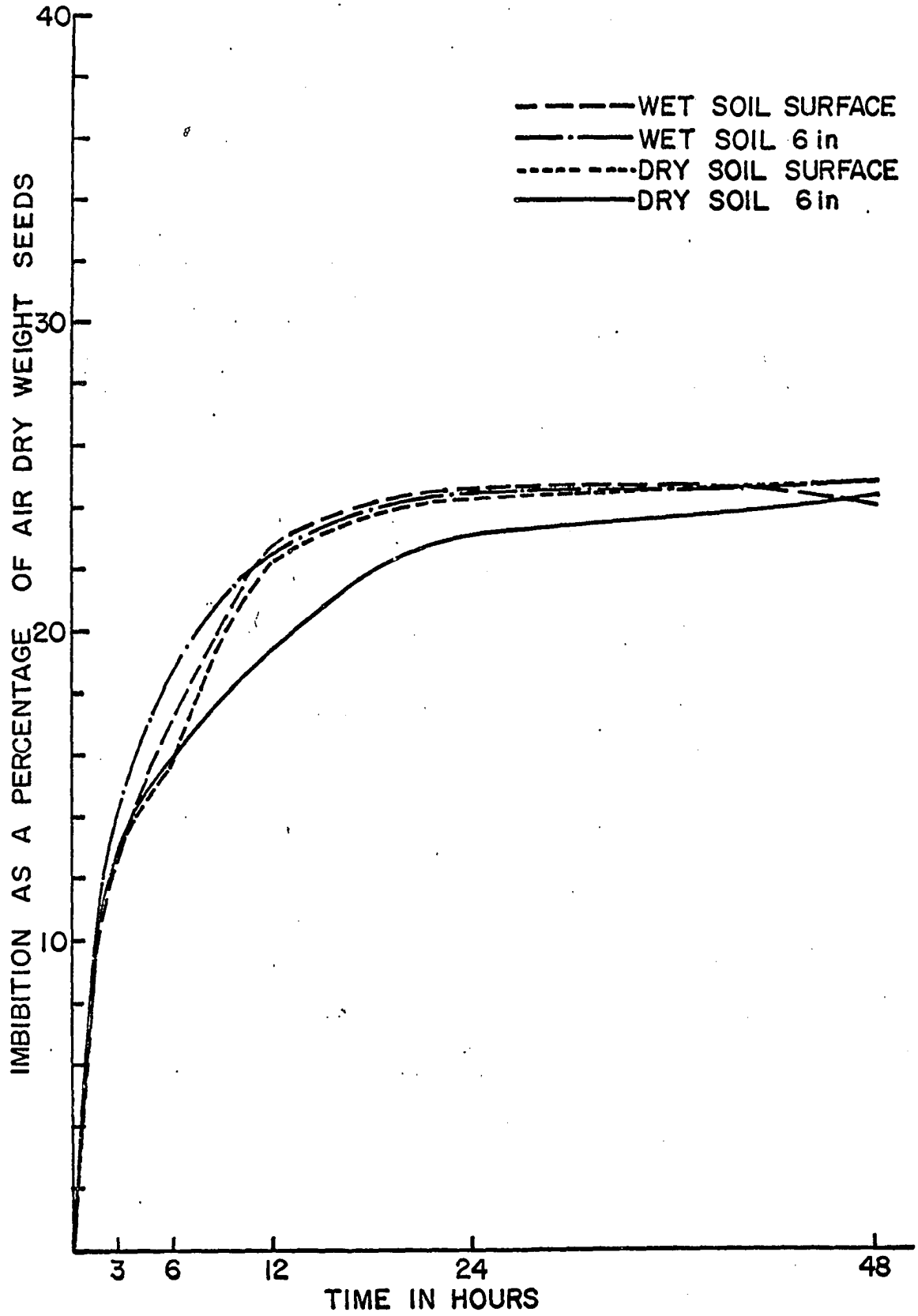
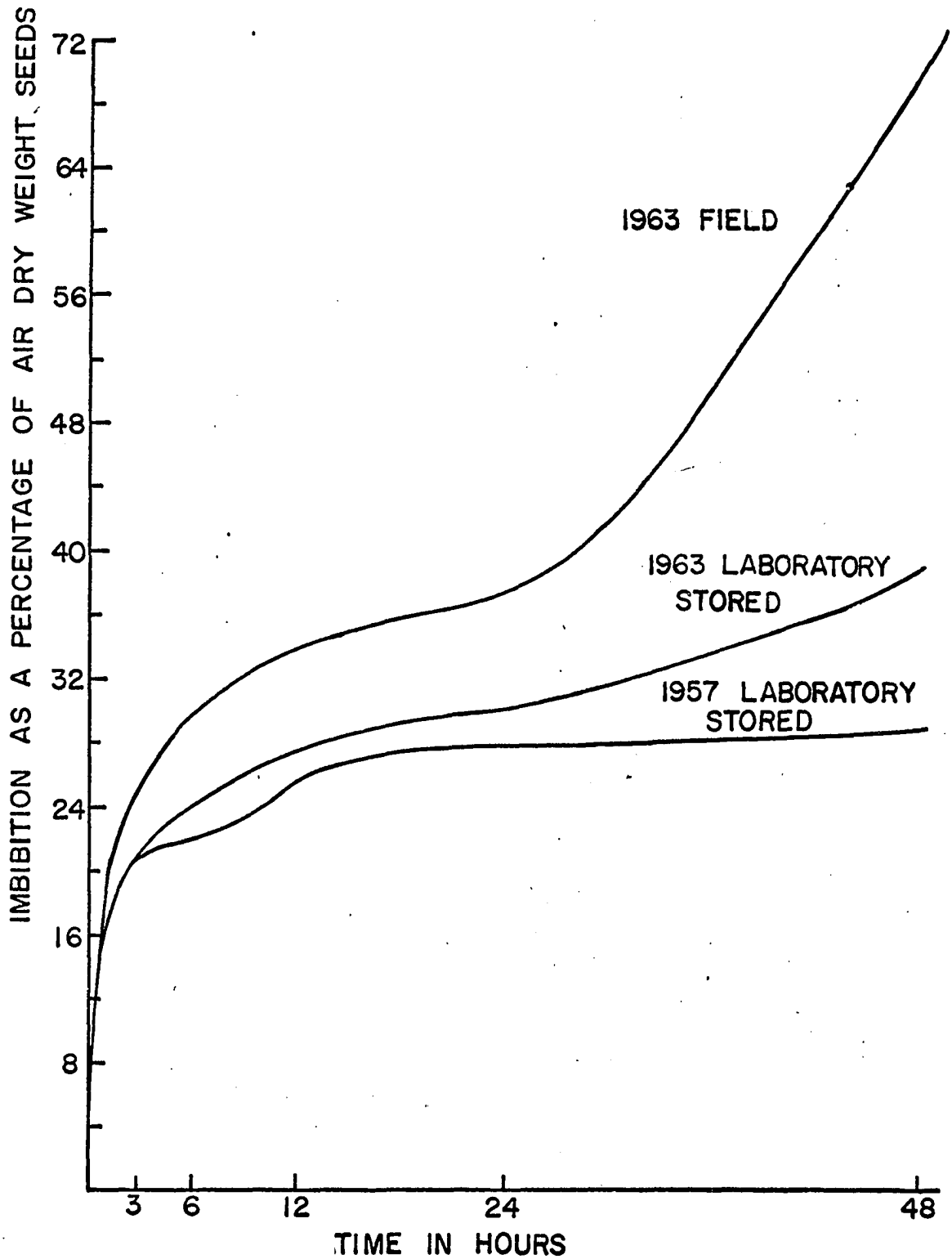

WET SOIL SURFACE
WET SOIL 6 in
DRY SOIL SURFACE
DRY SOIL 6 in
IMBIBITION AS A PERCENTAGE OF AIR DRY WEIGHT SEEDS
40
30
20
10
3
6
12
24
48
TIME IN HOURS

Figure 7. Water absorption curves for three lots of seed of Polygonum pensylvanicum with seed coats removed


IMBIBITION AS A PERCENTAGE OF AIR DRY WEIGHT SEEDS
72
64
56
48
40
32
24
16
8
1963 FIELD
1963 LABORATORY STORED
1957 LABORATORY STORED
3
6
12
24
48
TIME IN HOURS

Susceptibility to fumigation during after-ripening

Seed of Setaria lutescens and Polygonum pensylvanicum sampled from the field were fumigated to determine the susceptibility of seed to fumigant toxicity during the after-ripening process. On each sampling date a portion of the Setaria lutescens and Polygonum pensylvanicum seed taken from the soil were fumigated with 950 mg/l Vapam with exposures of 24 hours at 68°F. Germination data are summarized in Table 22. Germination of Setaria lutescens seed was not affected by Vapam fumigation until termination of dormancy occurred and after dormancy terminated germination of fumigated seed was greatly decreased. Seed of Polygonum pensylvanicum fumigated with Vapam did not show a reduction in germination, but germination was generally very poor and samples taken 20 or 22 weeks after burial were the only samples where reliable estimates of Vapam toxicity were obtained.

Viability of Polygonum pensylvanicum and Setaria lutescens seed fumigated with Vapam was investigated to determine toxicity of Vapam to after-ripened seed. Viability of seed was determined by culture of isolated embryos or germination of seed with seed coats removed. These results are summarized in Table 23. No decrease in viability of Setaria lutescens was observed with Vapam fumigation, however, embryos were isolated immediately after fumigation and from previously presented data these may not be a true representation of the effect of exposure to Vapam. Seed of Polygonum pensylvanicum did not show any effect of Vapam fumigation during 24 weeks of after-ripening. Fumigation of after-ripened Setaria lutescens and Polygonum pensylvanicum seed by allyl

Table 22. Germination percentages of Setaria lutescens and Polygonum pensylvanicum seed after-ripened in wet or dry soil at various depths and exposed to 950 mg/1 Vapam

Weeks of after-ripening	Wet soil depth, inches				Dry soil depth, inches			
	0	1	3	6	0	1	3	6
Setaria lutescens								
0	0	0	0	0	0	0	0	0
3	0	1/4	1/4	1	1	2	1	1
5	1	0	0	1/2	1	2	3	4
8	1/4	1/4	0	1/4	0	1/2	1	0
10	0	1	2	1/2	0	1/2	2	1
13	0	0	0	0	1	0	2	2
16	1/2	0	0	1	1	-	6	6
18	0	0	0	0	0	-	0	0
20	0	0	0	0	-	-	3	0
22	0	0	0	2	-	-	2	9
24	0	0	0	0	-	-	0	0
Polygonum pensylvanicum								
0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
10	1/2	0	0	0	0	0	0	0
13	1/2	0	1	0	0	0	0	1/2
16	0	0	0	0	0	0	0	0
18	0	5	2	0	0	1/2	1	0
20	0	10	8	0	-	-	1	0
22	5	6	0	9	-	-	6	17
24	1	0	0	0	-	-	0	0

Table 23. Germination percentages of excised embryos of Setaria lutescens and Polygonum pensylvanicum seed after-ripened in wet or dry soil at various depths and exposed to 950 mg/l Vapam

Weeks of after-ripening	Wet soil depth, inches				Dry soil depth, inches			
	0	1	3	6	0	1	3	6
Setaria lutescens								
0	80	80	80	80	80	80	80	80
3	60	20	20	0	0	0	40	20
5	80	70	30	90	-	-	-	-
8	20	80	0	0	100	80	0	100
10	0	40	0	40	20	80	0	0
13	80	60	80	60	50	-	70	60
16	75	65	60	55	95	-	95	95
18	90	90	100	100	50	-	90	100
20	80	100	100	80	-	-	100	100
22	100	90	100	60	-	-	70	70
24	80	80	80	70	-	70	90	70
Polygonum pensylvanicum								
0	100	60	70	100	85	100	90	80
3	75	75	60	55	80	80	65	70
5	30	50	60	60	60	70	80	60
8	100	60	40	100	100	50	40	100
10	90	90	90	80	80	80	90	80
13	80	60	60	90	80	90	90	70
16	100	100	100	100	70	-	70	100
18	50	80	70	100	20	-	80	80
20	80	60	60	70	-	-	70	60
22	70	70	80	50	-	-	70	100
24	70	30	60	40	-	-	60	60

alcohol, methyl bromide, and propylene oxide also demonstrated that the after-ripening process did not modify the response of the seed to each fumigant. These three fumigants killed both species before the seed were buried as well as throughout the after-ripening process.

Fumigation of seed in soil samples

Effect of fumigation on weed seed in the soil was investigated to determine the effect of soil on fumigant toxicity and seed susceptibility. Fumigated soil samples were soil samples with wild populations of weed seed or samples to which 50 seed of non-dormant Abutilon theophrasti and Setaria lutescens had been added. No non-dormant seed of Polygonum pensylvanicum were available and seed of Abutilon theophrasti were substituted. Soil used was Clarion loam and samples were in 6x6x2 inch wire baskets during fumigation. After fumigation the soil was uniformly distributed on Vermiculite in greenhouse flats for germination tests. Fumigation with 3800 mg/l Vapam resulted in 100 percent inhibition of germination of Setaria lutescens, Setaria viridis, Portulaca oleracea, Chenopodium album, and Amaranthus retroflexus from wild populations. No species were found that escaped control by Vapam fumigation at this concentration. Experiments conducted with known numbers of seed in each sample and fumigated with Vapam, allyl alcohol, and methyl bromide resulted in inhibition of germination of Setaria lutescens. Exposure of samples to Vapam and methyl bromide did not injure appreciably seed of Abutilon theophrasti. Lower concentrations of allyl alcohol did not inhibit germination of Abutilon theophrasti as completely in the presence of soil as when seed were in petri dishes. Table 24 summarizes

germination percentages of Setaria lutescens and Abutilon theophrasti fumigated in soil samples.

Table 24. Germination percentages of seed of Abutilon theophrasti and Setaria lutescens in soil exposed to three fumigants for 24 hours at 68°F

Fumigant	Concn. mg/l	Germination percentages	
		Setaria lutescens	Abutilon theophrasti
None	0	83	59
Vapam	4750	0	43
	1900	0	50
Allyl alcohol	870	0	1
	435	0	2
	145	0	17
	78	0	12
Methyl bromide	470	0	42

DISCUSSION

The responses of annual weed seeds to various soil fumigants, observed in this investigation, indicated the differential responses of dormant and non-dormant seed, showed the variation in susceptibility among weed species, revealed differences among the several fumigants in toxicity to weed seed, and demonstrated the increase in fumigant toxicity with increased exposure times. The patterns of germinability and dormancy observed with weed seeds after-ripened under field conditions, and the responses of these seeds to fumigants, showed the importance of moisture content of seed as a factor in susceptibility to fumigants, indicated a rather short period of maximum susceptibility of seed in the field, revealed that dormancy was not terminated at the same time for all weed species in the field, and suggested some difficulties in coordinating soil fumigation treatments with periods of maximum seed germinability in the field.

Dormant seed of Abutilon theophrasti were not killed by concentrations of 2800 mg/l methyl bromide, 1400 mg/l propylene oxide and 1900 mg/l Vapam, but exposure to 23 mg/l allyl alcohol gave nearly complete kill. Dormant seed of Polygonum pensylvanicum were killed by exposures to 470 mg/l methyl bromide, 700 mg/l propylene oxide and 23 mg/l allyl alcohol but not by exposure to Vapam. Isolated embryos of dormant seed of Setaria lutescens were killed by fumigation with methyl bromide, allyl alcohol, and propylene oxide, but not Vapam. Vapam fumigation appeared to be killing non-dormant and not dormant seed of Setaria lutescens. However, later studies showed that embryos of non-dormant

seed of Setaria lutescens were not killed by Vapam fumigation even though intact seed did not germinate. Apparent toxicity of Vapam to non-dormant and not dormant seed of Setaria lutescens was explained when embryos removed from each type immediately after fumigation showed no kill. Vapam fumigation of non-dormant seed of Setaria lutescens resulted in an inhibition of germination and eventual kill of intact seed in germinating conditions. The embryo was not killed until the seed had been in germinating conditions for several days. Clipping the lemma and palea so more rapid aeration of the caryopsis could occur did not reduce kill when seed were placed in germinating conditions. This suggested the fumigant was not trapped inside the seed and extending the length of exposure to the fumigant. Removal and germination of caryopses from treated seed anytime before the seed had been placed in germinating conditions resulted in germination of the caryopsis. Vapam fumigation of caryopses alone or embryos alone resulted in kill of the embryo in both instances. One must conclude that toxicity of Vapam to seed of Setaria lutescens was a complex interaction of fumigant, moisture, caryopsis, and lemma and palea.

Vapam was not toxic to any of the dormant seeds and allyl alcohol was toxic to all seeds. Abutilon theophrasti, which has a seed coat impermeable to water and gas, was the least susceptible of the dormant seeds and Polygonum pensylvanicum and Setaria lutescens in which dormancy is caused by germination inhibitors were readily susceptible. Toxicity to seed of Abutilon theophrasti was not dependent upon the vapor pressure of the fumigant as allyl alcohol at 68°F has a vapor pressure of 17.9 mm of mercury and Vapam, propylene oxide, and methyl bromide have vapor

pressures of 16.5, 440, and 1350 mm of mercury, respectively. Entrance of the fumigant into the seed was not on a pressure basis but must have been due to action of the fumigant.

Toxicity of soil fumigants to non-dormant weed seeds was dependent upon the physio-chemical condition of the seed and concentration of the fumigant. Vapam was quite toxic to seed of Setaria lutescens and Setaria faberi at 80 mg/l but even at extremely high concentrations, 1900 mg/l, dry seed of Abutilon theophrasti were not killed. At all concentrations of Vapam, dry seed were less susceptible than moist seed. A similar toxicity pattern was observed with propylene oxide. Allyl alcohol was extremely more toxic than either Vapam or propylene oxide and killed moist and dry seed of Abutilon theophrasti equally well at 11.6 mg/l. Moisture content of seed of Setaria lutescens and Setaria faberi became critical only at very low concentrations of allyl alcohol. Methyl bromide was toxic to all seeds except dry Abutilon theophrasti and appeared to be less toxic than allyl alcohol but more toxic than Vapam and propylene oxide. With all fumigants a greater concentration resulted in increased percent or increased rate of kill of weed seeds.

Fumigation of seed of Setaria faberi and Abutilon theophrasti in soil samples at similar concentrations as seed alone indicated a decrease in effectiveness of fumigants. Decrease in toxicity may have resulted from absorption of the fumigant on soil particles and solution in the soil water decreasing the effective concentration or from a lack of penetration of the fumigant into the soil. Allyl alcohol fumigation resulted in excellent control of seed of Setaria faberi and lesser control

of Abutilon theophrasti. Fumigation with Vapam and methyl bromide also gave excellent control of seed of Setaria faberi but very little control of Abutilon theophrasti. These relative patterns of kill are the same as those shown by the fumigants on seed alone. Results showed that all fumigants were getting to the seed as Setaria faberi were killed, but not in as great a concentration as without soil because allyl alcohol did not completely kill Abutilon theophrasti. The kill of seed of Abutilon theophrasti shown by Vapam and methyl bromide probably was due to rapid imbibition of water by some seed when placed in the soil.

Moisture content of seeds was important in determining the reactiveness and toxicity of fumigants and subsequent susceptibility of seed to kill by fumigation. Experiments designed to determine at what moisture content seeds became most susceptible to fumigation showed that this moisture percentage varied depending upon the fumigant used. With seed of Abutilon theophrasti each fumigant showed a different pattern of toxicity as moisture content increased. Propylene oxide had a gradual decrease in toxicity from 100 percent kill at about 40 percent moisture down to no kill at five percent moisture, whereas Vapam has a similar range of decrease in toxicity from 17 to five percent moisture. Methyl bromide had essentially no toxicity under nine percent moisture and almost 100 percent kill occurred at moisture percentages above ten percent. Seed moisture did not effect toxicity of allyl alcohol to seed of Abutilon theophrasti in these tests. Experiments with seed of Setaria lutescens again illustrated the importance of water content but the exact percentages needed for optimum effectiveness of fumigation were not obtained.

The tests did show the range to be somewhat less than that found for seed of Abutilon theophrasti as above 17 percent all fumigants were effective and again some were effective at moisture percentages of air dry seed. Relationships between moisture and toxicity bears out the known mode of action of each fumigant. Methyl bromide and allyl alcohol have a specific reaction that results in toxicity and when this system was activated the fumigant was readily toxic. Vapam has a non-specific reaction causing kill and as moisture content increased a corresponding increase in toxicity occurred. The additional water enhanced all reactions and no one specific reaction was responsible for the death of the seed. Overall, imbibed water that brought seed moisture into the range of 9-17 percent was the most critical as the most complete and rapid kill occurred in this range.

The importance of this range may be better understood when viewed in relation to seed moisture of seeds in the soil. Polygonum pensylvanicum and Setaria lutescens seed sampled from the soil during the winter and spring of 1963-64 had moisture percentages at the lower end of this range determined for Abutilon theophrasti and Setaria lutescens. These seeds would not be in their most susceptible condition and wetting the soil before treatment as recommended for best results with fumigation assumes importance in making the seeds more susceptible to fumigation with the end result better weed control. Tests made to determine at what soil moisture percentages seeds would imbibe water and germinate in Clarion loam, showed that seed of Abutilon theophrasti and Setaria lutescens germinated at 20 percent soil moisture whereas only Abutilon

theophrasti germinated at 16 percent and neither germinated at 12 or 8 percent. Some imbibition of water by seed of Abutilon theophrasti occurred at the two lower soil moistures but not sufficient for germination. These tests again showed the importance of wetting the soil before fumigation and for best results the water added should be sufficient to get the soil moisture near that of field capacity. Interaction of humidity in atmosphere surrounding the seed and seed susceptibility was determined by fumigating seed in various relative humidities at 68°F. This treatment does not include the soil factor but the importance of the high relative humidity found in soil air could be determined. Relative humidities of 100, 87, 50 and 25 percent had no effect on toxicity to seed of Abutilon theophrasti and Setaria lutescens. Thus seed moisture was very important if not the most important factor in affecting seed susceptibility to soil fumigation in the laboratory.

The suggestion that soil decreases the effectiveness of fumigation increases the importance of fumigating when the seeds are most susceptible to kill. Effect of seed moisture on susceptibility has shown that with increased moisture there was an increase in susceptibility. An increase in moisture content suggests that the germination process has begun and, therefore the optimum time for weed control by fumigation would be just as germination and seedling development occur in the field. Studies with seed of Polygonum pensylvanicum and Setaria lutescens from the field showed that dormancy with seed of Setaria lutescens had terminated by the end of January and maximum germination had occurred in the field by the end of April. Dormancy with seed of Polygonum pensylvanicum did not terminate until immediately before germination and seeds from 1-3 inches

in the soil had 70-90 percent germination by mid-April. After germination seedling development proceeded rapidly and emergence of Setaria lutescens began in late April and reached a maximum by mid-May. Polygonum pensylvanicum emergence also occurred in late April and had a maximum by mid-June. From these studies the optimum time for field fumigation would be late April or early May. At this time seeds would be most susceptible as the germination process would have begun and yet seedlings would not be so well established as to allow the plant to escape.

Exposure time of seed to the fumigant is important in weed control and also reveals further understanding of the toxicity of the fumigants. The concentrations of Vapam, methyl bromide, and allyl alcohol were in excess of those calculated from recommended rates for equal volumes of soil so that the length of time needed for kill in the field would probably be longer. Exposure to Vapam for lengths of time 1 hour or less usually did not injure the seed. Between 1 and 3 hours a large increase in kill occurred and after 24 hours all seeds were killed except intact Polygonum pensylvanicum and dry treatments of Abutilon theophrasti. Fumigation time with propylene oxide gave a toxicity pattern similar to Vapam as exposure time increased from 15 minutes to 3 hours a gradual increase in seed kill occurred. Methyl bromide showed a rapid kill, 15 minutes in many treatments, and allyl alcohol was faster still as moist seed of Abutilon theophrasti and Polygonum pensylvanicum and treatments with seed coats removed were killed in 5 minutes and all other seeds were killed in 30 minutes.

Comparison of germination of treated intact seed of Polygonum pensylvanicum and Abutilon theophrasti and those with seed coats removed

showed the effect of the seed coats on movement and action of Vapam into these seeds. Abutilon theophrasti seed coats reduced the rate of kill very slightly in moist treatments and had no effect on dry treated seed. Seed coats of Polygonum pensylvanicum had a definite effect on susceptibility to Vapam as intact seed were not harmed by 24 hour fumigation and with seed coats removed they were almost completely killed in 3 hours. The two seed coats are somewhat different in that Abutilon theophrasti has a true seed coat with a thick waxy cuticle and a lignified and cutinized palisade layer underneath, whereas in Polygonum pensylvanicum the seed coat that was removed was actually a hard thick fruit coat of the achene. Fumigation with methyl bromide and propylene oxide illustrated a impeding effect of the seed coats of Abutilon theophrasti and Polygonum pensylvanicum. The rate of kill was faster with seed coats removed but the degree was the same. Toxicity of allyl alcohol was not affected by the seed coats as all seed were killed in relatively short periods of time.

Data from exposure studies often showed treated seeds to have greater germination than untreated seeds. Conditions under which increased germination of treated seed was observed could not be determined precisely and thus was not readily reproducible, but it did occur in enough replications to warrant consideration. If these data do represent an actual stimulation in germination caused by fumigation it is possibly an increased activity just prior to kill and not a true stimulation. If it were more than just increased activity before death the low concentrations that did not kill the seed should suggest signs of stimulation and this phenomenon did not occur.

From the present study the order of fumigant toxicity on these species from greatest to least would be allyl alcohol, methyl bromide, propylene oxide and Vapam. This is not an indication of the toxicity of the active ingredient of each because reagent grade allyl alcohol and propylene oxide were used and methyl bromide was 98 percent methyl bromide and 2 percent chloropicrin and Vapam was 32.8 percent methyl dethiocarbamate. As presented previously, toxicity did not appear dependent upon the vapor pressure as they are 1350, 440, 17.9, and 16.5 mm of mercury at 68°F for methyl bromide, propylene oxide, allyl alcohol and Vapam, respectively. Degree of toxicity of these fumigants appeared to depend upon how specific its mode of action is.

Future experiments to develop soil fumigation as a weed control practice would have to utilize known information on toxicity of the various fumigants to seeds and the susceptibility of seeds in various conditions. In addition more precise information of the condition of the seed in the soil would have to be obtained to determine the period of maximum susceptibility to kill by fumigation. This would entail a detailed study of dormancy termination, moisture uptake, germination, seedling development and environmental conditions associated with the seed during these various processes. The possibility of developing a method of concentrating weed seed germination into a short time period in the spring should be explored as a means of increasing the efficiency of soil fumigation as a weed control practice. Also, the development of a more efficient fumigant and one that has a broader toxicity base must be considered.

SUMMARY

Soil fumigants were toxic to annual weed seed. Toxicity patterns of the several fumigants were dependent upon the basic toxicity of the fumigant and susceptibility of the weed seed. Exposure to the experimental fumigants readily inhibited germination of seed of Setaria lutescens and Setaria faberi. Generally, non-dormant seed were more susceptible to kill with fumigation than dormant seed. Dormant seed of Abutilon theophrasti were least affected by fumigation of the weed species in this study.

Toxicity of the several fumigants to weed seed increased with increased length of exposure of the seed to the fumigant, with increased moisture content of the seed, and with increased concentration of the fumigant. Allyl alcohol was toxic to several seed with exposures of five minutes and all seed susceptible to the experimental fumigants were killed with exposures of 24 hours. With exposure to each fumigant a different seed moisture at which 90 percent of non-dormant seed of Abutilon theophrasti failed to germinate was observed. With allyl alcohol, methyl bromide, Vapam, and propylene oxide this seed moisture percentage was 5, 9.5, 17, and 40 percent, respectively. With the several fumigants and weed species, there was no discernible increase in toxicity with an increase in temperature during exposure of seed to fumigants.

Toxicity patterns observed by failure of weed seed to germinate showed allyl alcohol to be the most toxic followed in order of decreasing toxicity by methyl bromide, propylene oxide, and Vapam. Unique with

Vapam fumigation was the observed inhibition of germination of intact seed of Setaria lutescens, but the kill of the embryo was not observed until the intact seed had been moistened.

The pattern of seed dormancy observed with seed of Setaria lutescens and the effectiveness of natural exposure in terminating dormancy suggested the cool, moist autumn climate promoted termination of dormancy conditioned by the caryopsis and dormancy maintained by lemma and palea was terminated after low temperature stratification during the winter. Dormancy observed with seed of Polygonum pensylvanicum also suggested the importance of autumn and winter conditions during after-ripening plus the alternating temperatures observed in the spring when dormancy terminated. With both species dormancy was terminated faster the deeper the seed were in the soil. Germination in the field occurred in a similar pattern as termination of dormancy, whereas seedling emergence was observed from the 1 or 3 inch depths significantly more than from the surface or 6 inch depth. The patterns of seed dormancy observed with seed of Setaria lutescens and Polygonum pensylvanicum under natural conditions suggested a relatively short period of maximum susceptibility to fumigation. Utilization of soil fumigation as a superior weed control practice would require detailed information on the physiological state of the weed seed to coordinate fumigant treatment with periods of maximum susceptibility.

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